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(57) Abstract

The present invention relates to novel human proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells and recombinant methods for producing the proteins of the invention. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human secreted proteins.

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Human Proteins

Field of the Invention

The present invention relates to genes encoding novel human proteins which exhibit a variety useful biological activities. More specifically, isolated nucleic acid molecules are provided which encode polypeptides comprising various forms of human proteins. Human polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. Also provided are methods for detecting nucleic acids or polypeptides related to those of the invention, for example, to aid in identification of a biological sample or diagnosis of disorders related to expression of protein genes of this invention. The invention further relates to methods for identifying agonists and antagonists of the proteins of the invention, as well as to methods for treatment of disorders related to protein gene expression using polypeptides, antagonists and agonists of the invention.

Background of the Invention

Identification and sequencing of human genes is a major goal of modern scientific research. For example, by identifying genes and determining their sequences, scientists have been able to make large quantities of valuable human gene products. These include human insulin, interferon, Factor VIII, human growth hormone, tissue plasminogen activator, erythropoeitin and numerous other proteins. Additionally, knowledge of gene sequences can provide keys to diagnosis, treatment or cure of genetic diseases such as muscular dystrophy and cystic fibrosis.

Despite the great progress that has been made in recent years, only a small number of genes which encode the presumably thousands of human proteins have been identified and sequenced. Therefore, there is a need for identification and characterization of novel human proteins and corresponding genes which can play a role in detecting, preventing, ameliorating or correcting disorders related to abnormal expression of and responses to such proteins.

Summary of the Invention

The present invention provides isolated nucleic acid molecules comprising polynucleotide sequences which have been identified as sequences encoding human proteins of the invention. Each protein of the invention is identified in Table 1, below (see Example 2) by a reference number designated as a "Protein ID (Identifier)" (e.g., "PF353-01"). Each protein of the invention is related to a human complementary DNA (cDNA) clone prepared from a messenger RNA (mRNA) encoding the related protein. The cDNA clone related to each protein of the invention is identified by a "cDNA Clone ID (Identifier)" in Table 1 (e.g., "HABCE99"). DNA of each cDNA clone in Table 1 is contained in the material deposited with the American Type Culture Collection and given the ATCC Deposit Number shown for each cDNA Clone ID in Table 1, as further described below.

The invention provides a nucleotide sequence determined for an mRNA molecule encoding each protein identified in Table 1, which is designated in Table 1 as the "Total NT (Nucleotide) Sequence." This determined nucleotide sequence has been assigned a SEQ ID NO = "X" in the Sequence Listing hereinbelow, where the value of X for the determined nucleotide sequence of each protein is an integer specified in Table 1. The determined nucleotide sequence provided for each protein of the invention was determined by applying conventional automated nucleotide sequencing methods to DNA of the corresponding deposited cDNA clone cited in Table 1.

The determined nucleotide sequence for the mRNA encoding each protein of the invention has been translated to provide a determined amino acid sequence for each protein which is identified in Table 1 by a SEQ ID NO = "Y" where the value of Y for each protein is an integer defined in Table 1. The determined amino acid sequence for each protein represents the amino acid sequence encoded by the determined nucleotide sequence, beginning at or near the translation initiation ("start") codon of the protein and continuing until the first translation termination ("stop") codon. Due to possible errors inherent in determining nucleotide sequences from any DNA molecule, particularly using the conventional automated sequencing technology used

to sequence the cDNA clones described herein, occasional nucleotide sequence errors are expected in the determined nucleotide sequences of the invention. These errors may include insertions or deletions of one or a few nucleotides in the determined nucleotide sequence as compared to the actual nucleotide sequence of the deposited cDNA. As one of ordinary skill would appreciate, incorrect insertions or deletions of one or two nucleotides into a determined nucleotide sequence leads to a shift in the translation reading frame compared to the reading frame actually encoded by a cDNA clone. Further, such a shift in frame within an actual open reading frame frequently leads to the appearance of a translation termination (stop) codon within the sequence encoding the polypeptide. Accordingly, due to occasional errors in the nucleotide sequences determined from the deposited cDNAs and any related DNA clones used to prepare the determined sequence for the mRNA encoding each secreted protein of the invention, the translations shown as determined amino acid sequences in SEQ ID NO:Y may represent only a portion of the complete amino acid sequence of the human secreted protein actually encoded by the mRNA represented by the corresponding cDNA clone in the ATCC deposit identified in Table 1. In any event, the determined amino acid sequence for each protein in Table 1, which is shown in SEQ ID NO:Y for each protein, comprises at least a portion of the amino acid sequence determined for that protein.

More particularly, the determined amino acid sequence is the amino acid sequence translated from the determined nucleotide sequence in the open reading frame of the first amino acid of the ORF to the last amino acid of that frame. In other words, the determined amino acid sequence is translated from the determined nucleotide sequence beginning at the codon having as its 5' end the nucleotide in the position of SEQ ID NO:X identified in Table 1 as the 5' nucleotide of the first amino acid (abbreviated in Table 1 as "5' NT of First AA"). Translation of the determined nucleotide sequence is continued in the reading frame of that first amino acid codon to the first stop codon in that same open reading frame, i.e., to the position in SEQ ID

NO:X which encodes the amino acid at the position in SEQ ID NO:Y identified as the "last amino acid of the open reading frame" (abbreviated as "Last AA of ORF").

For any determined amino acid sequence in which the first amino acid is the methionine encoded by the translation initiation codon for the protein, Table 1 also identifies the position in SEQ ID NO:X of the 5' nucleotide of the start codon ("5' NT of Start Codon") as the same position in SEQ ID NO:X as that of the 5' nucleotide of the first amino acid ("First AA").

Table 1 also identifies the positions in SEQ ID NO:Y of the last amino acid of the signal peptide ("Last AA of Sig Pep") and the first amino acid of the secreted portion ("First AA of Secreted Portion") of the protein, for those polypeptide having a secretory leader sequence. The "secreted portion" of a secreted protein in the present context indicates that portion of the complete polypeptide translated from an mRNA which remains after cleavage of the signal peptide by a signal peptidase. In this context the term "mature" may also be used interchangeably with "secreted portion" although it is recognized that in other contexts "mature" may designate a portion of a "proprotein" which is produced by further cleavage of the polypeptide after cleavage of the signal peptide.

Accordingly, in one aspect the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence which is identical to the nucleotide sequence of SEQ ID NO:X, where X is any integer as defined in Table 1. The invention also provides an isolated nucleic acid molecule comprising a nucleotide sequence which is identical to a portion of the nucleotide sequence of SEQ ID NO:X, for instance, a sequence of at least 50, 100 or 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X. Such a portion of the nucleotide sequence of SEQ ID NO:X may be described most generally as a sequence of at least C contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X where: (1) the sequence of at least C contiguous nucleotides begins with the nucleotide at position N of SEQ ID NO:X and ends with the nucleotide at position M of SEQ ID NO:X; (2) C is any integer in the range beginning with a convenient primer size, for instance, about 20, to

the total nucleotide sequence length ("Total NT Seq.") as set forth for SEQ ID NO:X in Table 1; (3) N is any integer in the range of 1 to the first position of the last C nucleotides in SEQ ID NO:X, or more particularly, N is equal to the value of Total NT Seq. minus the quantity C plus 1 (i.e., Total NT Seq.–(C+1)); and (4) M is any integer in the range of C to Total NT Seq.

Preferably, the sequence of contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X is included in SEQ ID NO:X in the range of positions beginning with the nucleotide at about the 5' nucleotide of the clone sequence ("5' NT of Clone Seq." in Table 1) and ending with the nucleotide at about the 3' nucleotide of the clone sequence ("3' NT of Clone Seq." in Table 1). More preferably, the sequence of contiguous nucleotides is in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Start Codon ("5' NT of Start Codon" in Table 1) and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as set forth for SEQ ID NO:X in Table 1. For instance, one preferred embodiment of this aspect of the invention is an isolated nucleic acid molecule which comprises a sequence at least 95%, 96%, 97%, 98%, or 99% identical to a sequence of about 500 contiguous nucleotides included in the nucleotide sequence of SEQ ID NO:X beginning at about the 5' NT of Start Codon position as set forth for SEQ ID NO:X in Table 1. Another preferred embodiment of this aspect of the invention is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X beginning with the nucleotide at about the position of the 5' Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Further embodiments of the invention include isolated nucleic acid molecules which comprise a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98%, 99% or 99.9% identical, to any of the determined nucleotide sequences above. For instance, one such embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a

sequence of at least 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1. Another embodiment of this aspect of the invention is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X.

Isolated nucleic acid molecules which hybridize under stringent hybridization conditions to a nucleic acid molecule described above also are provided. Such a nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

The invention further provides a composition of matter comprising a nucleic acid molecule which comprises a human cDNA clone identified by a cDNA Clone ID (Identifier) in Table 1, which DNA molecule is contained in the material deposited with the American Type Culture Collection and given the ATCC Deposit Number shown in Table 1 for that cDNA clone. As described further in Example 1, this deposited material comprises a mixture of plasmid DNA molecules containing cloned cDNAs of the invention. Further, the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence which is, for instance, at least 95% identical to a sequence of at least 50, 150 or 500 contiguous nucleotides in the nucleotide sequence encoded by a human cDNA clone contained in the deposit given the ATCC Deposit Number shown in Table 1. One preferred embodiment of this aspect is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by a human cDNA clone identified in Table 1 and as contained in the deposit with the ATCC Deposit Number shown in Table 1. Also provided are isolated nucleic acid molecules which hybridize under stringent hybridization conditions to a nucleic acid molecule comprising a nucleotide sequence encoded by a human cDNA clone identified in Table 1 and contained in the cited deposit.

These nucleic acid molecules of the invention may be used for a variety of identification and diagnostic purposes. For instance, the invention provides a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a nucleotide sequence of the invention. The sequence of the nucleic acid molecule used in this method is selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. This method of the invention comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in the biological sample with a sequence selected from the group above, and determining whether the sequence of the nucleic acid molecule in the sample is at least 95% identical to the selected sequence. The step of comparing sequences may comprise determining the extent of nucleic acid hybridization between nucleic acid molecules in the sample and a nucleic acid molecule comprising the sequence selected from the above group. Alternatively, this step may be performed by comparing the nucleotide sequence determined from a nucleic acid molecule in the sample, for instance by automated DNA sequence methods, with the sequence selected from the above group.

In another aspect, the invention provides methods for identifying the species, tissue or cell type of a biological sample based on detecting nucleic acid molecules in the sample which comprise a nucleotide sequence of a nucleic acid molecule of the invention (for instance, a nucleic acid molecule comprising a nucleotide sequence that is at least 95% identical to at least a portion of a nucleotide sequence of SEQ ID NO:X or a nucleotide sequence encoded by a human cDNA clone identified in Table 1 as contained in the deposit with the ATCC Deposit Number shown therein. This method may be conducted by detecting a nucleotide sequence of an individual cDNA of the invention or using panel of nucleotide sequences of the invention. Thus, this method may comprise a step of detecting nucleic acid molecules comprising a

nucleotide sequence in a panel of at least two nucleotide sequences, where at least one sequence in the panel is at least 95% identical to at least a portion of a nucleotide sequence of SEQ ID NO:X or a nucleotide sequence encoded by a human cDNA clone contained in the ATCC deposit. In this method for identifying the species, tissue or cell type of a biological sample, the detection of nucleic acid molecules comprising nucleotide sequences of the invention may be conducted by various techniques known in the art including, for instance, hybridization of either DNA or RNA probes to either DNA or RNA molecules obtained from the biological sample, as well as computational comparisons of nucleotide sequences determined from nucleic acids in a biological sample with nucleotide sequences of the invention.

Similarly, nucleic acid molecules of the invention may be used in a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a protein identified in Table 1. This method may comprise a step of detecting in a biological sample obtained from the subject nucleic acid molecules comprising a nucleotide sequence that is at least 95% identical to at least a portion of a nucleotide sequence of SEQ ID NO:X or a nucleotide sequence encoded by a human cDNA clone identified in Table 1 as contained in the deposit with the given ATCC Deposit Number. Again, this diagnostic method may involve analysis of individual nucleotide sequences or panels of several nucleotide sequences, and the analysis of either DNA or RNA species using either DNA or RNA probes.

For use in identification or diagnostic methods such as those described above, therefore, the invention also provides a composition of matter comprising isolated nucleic acid molecules in which the nucleotide sequences of the nucleic acid molecules comprise a panel of sequences, at least one of which is at least 95% identical to a sequence, either a nucleotide sequence of SEQ ID NO:X or a nucleotide sequence encoded by a human cDNA clone contained in the ATCC deposit in Table 1. In this composition, the nucleic acid molecules may comprise DNA molecules or RNA molecules or both, as well as polynucleotide equivalents of DNA and RNA which are not naturally occurring but are known in the art as such.

Another aspect of the invention relates to polypeptides comprising amino acid sequences encoded by nucleotide sequences of the invention. For identification and diagnostic purposes, these polypeptides need not include the amino acid sequence of a complete secreted protein or even of the secreted form of such a protein, since, for instance, antibodies may bind specifically to a linear epitope of a polypeptide which comprises as few as 6 to 8 amino acids. Accordingly, the invention also provides an isolated polypeptide comprising an amino acid sequence at least 90%, preferrably 95%, 96%, 97%, 98%, or 99% identical to a sequence of at least about 10, 30 or 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1. Preferably, the sequence of contiguous amino acids is included in the amino acid sequence of SEQ ID NO:Y beginning with the residue at about the position of the First Amino Acid of the Secreted Portion where one exists or the first amino acid of the open reading frame if the protein is not indicated as having a signal peptide and ending with the residue at about the Last Amino Acid of the Open Reading Frame as set forth for SEQ ID NO:Y in Table 1. A preferred embodiment of this aspect relates to an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y.

As noted above, however, the determined amino acid sequence of SEQ ID NO:Y may not include the complete amino acid sequence of the protein encoded by each cDNA in the ATCC deposit identified in Table 1. Accordingly, the invention further provides an isolated polypeptide comprising an amino acid sequence at least 90% identical, preferrably at least 95%, 96%, 97%, 98% or 99% identical to a sequence of at least about 10, 300 or 100 contiguous amino acids in the complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for that cDNA clone in Table 1. A particularly preferred embodiment of this aspect is a polypeptide in which the sequence of contiguous amino acids is included in the amino acid sequence of a secreted ("mature") portion of the protein encoded by a human cDNA clone contained in the deposit, particularly a

polypeptide comprising the entire amino acid sequence of the secreted portion of the secreted protein encoded by a human cDNA clone of the invention.

For purposes such as tissue identification and diagnosis of pathological conditions, the invention also provides an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence of the invention, (for instance, a sequence that is identical to a sequence of at least 6, preferrably at least 7, 8, 9 or 10, contiguous amino acids in an amino acid sequence of SEQ ID NO:Y or in a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit cited therein. Further in the same vein, the invention provides a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is identical to a sequence of at least 6, preferrably at least 7, 8, 9 or 10 contiguous amino acids in a sequence selected from the group consisting of an amino acid sequence of SEQ ID NO:Y and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for that cDNA clone in Table 1;. This method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from the above group and determining whether the sequence of that polypeptide molecule in the sample is identical to the selected sequence of at least 6-10 contiguous amino acids. This step of comparing an amino acid sequence of at least one polypeptide molecule in the sample with a sequence selected from the above group may comprise determining the extent of specific binding of polypeptides in the sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence of the invention. Alternatively, this comparison step may be performed by comparing the amino acid sequence determined from a polypeptide molecule in the sample with the sequence selected from the above group, for instance, using computational methods.

The invention further provides methods for identifying the species, tissue or cell type of a biological sample comprising a step of detecting polypeptide molecules

in the sample which include an amino acid sequence that is identical to a sequence of at least 6-10 contiguous amino acids an amino acid sequence of SEQ ID NO:Y or of a cDNA identified in Table 1 and contained in the cited deposit. This method may involve analyses of polypeptides for the presence of individual amino acid sequences of the invention or of panels of such sequences. Similarly provided are methods for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a protein identified in Table 1. In preferred embodiments of these methods of the invention for identification or diagnosis, an antibody which binds specifically to a polypeptide comprising an amino acid sequence of the invention is used to analyze amino acid sequences of polypeptides in a biological sample.

In yet another aspect, the invention provides recombinant means for making a polypeptide comprising all or a portion of an amino acid sequence of the invention. For this purpose, an isolated nucleic acid molecule comprising a nucleotide sequence which is, for instance, at least 95% identical to a nucleotide sequence encoding a polypeptide which comprises an amino acid sequence of the invention (for instance, one that is at least 90% identical to SEQ ID NO:Y.

It will be readily appreciated by one of ordinary skill that, due to the degeneracy of the genetic code, any nucleotide sequence encoding the amino acid sequence of a given protein needs to share only a low level of identity with the nucleotide sequence of a human cDNA clone which encodes the identical amino acid sequence of that protein. It will be further appreciated that the nucleotide of the deposited cDNAs presumably all comprise codons optimized for expression by human cells from which the cDNAs originated. Therefore, for improved expression in recombinant prokaryotic host cells, for instance, it may be desirable to alter the codon usage in a nucleic acid molecule encoding an amino acid sequence of the invention, selecting codons in accordance with the redundancy of the genetic code, which provide optimal codon usage in the selected host. Preferred nucleic acid molecules of this aspect of the invention are those which encode a polypeptide which comprises an

complete amino acid sequence of SEQ ID NO:Y or a complete amino acid sequence of a protein encoded by a human cDNA clone identified in Table 1 and contained in the deposit cited therein.

Using such nucleic acid molecules encoding polypeptides of the invention, the invention further provides recombinant means for making the polypeptides. Thus, included is a method of making a recombinant vector comprising inserting an isolated nucleic acid molecule of the invention into a vector, as well as a recombinant vector produced by this method. Also included is a method of making a recombinant host cell comprising introducing a vector of the invention into a host cell, and a recombinant host so made. Such cells are useful, for instance, in a method of making an isolated polypeptide of the invention which comprises culturing a recombinant host cell under conditions such that the polypeptide is expressed and recovering the polypeptide.

In a preferred embodiment of this method, the recombinant host cell is a eukaryotic cell and the polypeptide encoded by the nucleic acid of the invention encodes the complete amino acid sequence of a protein encoded by a cDNA identified in Table 1, so that the polypeptide produced by this method is a secreted ("mature") portion of a human secreted protein of the invention (i.e., one comprising an amino acid sequence of SEQ ID NO:Y beginning with the residue at the position identified n Table 1 as the First AA of Secreted Portion of SEQ ID NO:Y or an amino acid sequence of a secreted portion of a secreted protein encoded by a human cDNA clone identified in Table 1 and contained in the deposit with the ATCC Deposit Number shown in Table 1. The invention further provides an isolated polypeptide which is a secreted portion of a human secreted protein produced by the above method. Where the polypeptide shown in Table 1 does not have a leader sequence one may be provided by the vector. Such vectors are known in the art and are discussed below.

In yet another aspect, the invention provides a method of treatment of an individual in need of an increased level of a secreted protein activity. As described herein, diagnostic methods of the invention enable the identification of such individuals, that is, individuals with a pathological condition involving a particular

organ, tissue or cell type, exhibiting lower levels of expression product (e.g., mRNA or antigen) of a given secreted protein in that organ, tissue or cell type, or those with mutant expression products, compared with normal individuals not suffering from the pathology. The method of the invention for treatment of an individual with such a pathological condition comprises administering to such an individual a pharmaceutical composition comprising an amount of an isolated polypeptide of a secreted protein of the invention effective to increase the level of activity of that secreted protein in the individual.

Agonists and antagonists of the polypeptides of the invention and methods for using these also are provided.

Brief Description of the Drawings

Figure 1 shows the nucleotide sequence and deduced amino acid sequence of CCV (HEMFI85), SEQ ID NOS:1 and 2, respectively.

Figure 2 shows the nucleotide sequence and deduced amino acid sequence of CAT-1 (HTXET53), SEQ ID NOS:3 and 4, respectively.

Figure 3 shows the nucleotide sequence and deduced amino acid sequence of CAT-2 (HT3SG28), SEQ ID NOS:5 and 6, respectively.

Figure 4 shows the nucleotide sequence and deduced amino acid sequence of MIA-2 (HBXAK03), SEQ ID NOS:7 and 8, respectively.

Figure 5 shows the nucleotide sequence and deduced amino acid sequence of MIA-3 (HLFBD44), SEQ ID NOS:9 and 10, respectively.

Figure 6 shows the nucleotide sequence and deduced amino acid sequence of AIF-2 (HEBGM49), SEQ ID NOS:11 and 12, respectively.

Figure 7 shows the nucleotide sequence and deduced amino acid sequence of AIF-3 (HNGBH45), SEQ ID NOS:13 and 14, respectively.

Figure 8 shows the nucleotide sequence and deduced amino acid sequence of Annexin (HSAAL25), SEQ ID NOS:15 and 16, respectively.

Figure 9 shows the nucleotide sequence and deduced amino acid sequence of ES/130-like I (HUSAX55), SEQ ID NOS:17 and 18, respectively.

Figure 10 shows the nucleotide sequence and deduced amino acid sequence of BEF (HSXCK41), SEQ ID NOS:19 and 20, respectively.

Figure 11 shows the nucleotide sequence and deduced amino acid sequence of ADF (HFKFY79), SEQ ID NOS:21 and 22, respectively.

Figure12 shows the nucleotide sequence and deduced amino acid sequence of Bcl-like (HAICH28), SEQ ID NOS:23 and 24, respectively.

Detailed Description

Nucleic Acid Molecules

Nucleotide Sequences and ATCC Deposits of cDNA Clones Encoding Human Proteins

The present invention provides isolated nucleic acid molecules comprising polynucleotide sequences which have been identified as sequences encoding human proteins. The invention further provides a nucleotide sequence determined from an mRNA molecule encoding each human protein identified in Table 1, which comprises all or a substantial portion of the complete nucleotide sequence of the mRNA encoding each protein of the invention and has been assigned a SEQ ID NO = "X" in the Sequence Listing and Figures hereinbelow,

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring nucleic acid molecule or polynucleotide present in a living organism is not isolated, but the same nucleic acid molecule or polynucleotide, separated from some or all of the coexisting materials in the natural environment, is isolated. Such nucleic acid molecule could be part of a vector and/or such polynucleotide could be part of a composition, and still be isolated in that such vector or composition is not part of the natural environment of the nucleic acid molecule or polynucleotide.

By "nucleotide sequence" of a nucleic acid molecule or polynucleotide is intended, for a DNA molecule or polynucleotide, a sequence of deoxyribonucleotides,

and for an RNA molecule or polynucleotide, the corresponding sequence of ribonucleotides (A, G, C and U), where each thymidine deoxyribonucleotide (T) in the specified deoxyribonucleotide sequence is replaced by the ribonucleotide uridine (U).

Using the information provided herein, such as a nucleotide sequence shown in the sequence listing, a nucleic acid molecule of the present invention encoding a polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. The present invention provides not only the determined nucleotide sequences of the mRNA encoding each human secreted protein of the invention, as set forth in SEQ ID NO:X for each protein, but also a sample of plasmid DNA containing a cDNA of the invention deposited with the American Type Culture Collection (Rockville, MD), as set forth in Table 1. These deposits enable recovery of each cDNA clone and recombinant production of each secreted protein of the invention actually encoded by a cDNA clone identified in Table 1, as further described hereinbelow.

Nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

In addition to nucleic acid molecules comprising a determined nucleotide sequence in SEQ ID NO:X or the nucleotide sequence of a deposited human cDNA clone, isolated nucleic acid molecules of the invention include DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode the proteins shown in the sequence listing or those encoded by the clones contained in the deposited plasmids. Of course, the genetic code and species-specific codon preferences are well known in the art. Thus, it would be routine for one skilled in the art to generate the degenerate variants described above, for instance, to optimize codon expression for a particular host (e.g.,

change codons in the human mRNA to those preferred by a bacterial host such as E. coli). Preferably, this nucleic acid molecule will encode a secreted portion (mature polypeptide) encoded by the deposited cDNA.

The invention further provides a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by in situ hybridization with chromosomes, and for detecting expression of the corresponding gene(s) in human tissue, for instance, by Northern blot analysis.

The present invention is further directed to nucleic acid molecules encoding portions of the nucleotide sequences described herein as well as to fragments of the isolated nucleic acid molecules described herein. By a "fragment" of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNA or the nucleotide sequence shown in the sequence listing is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 50-500 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNA or as shown in the sequence listing. By a fragment "at least 20 nt in length," for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the deposited cDNA or the determined nucleotide sequence shown in SEQ ID NO:X. Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding epitope-bearing portions of the polypeptides of the present invention, as described further below.

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of a nucleic acid molecule of the invention described above, for instance, a cDNA contained in the plasmid sample deposited with the ATCC. By "stringent hybridization conditions" is intended overnight incubation at 42° C in a

solution comprising: 50% formamide, 5x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65° C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 (e.g., 50) nt of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below. For certain applications, such as the FISH technique for gene mapping on chromosomes, probes of 500 nucleotides up to 2000 nucleotides may be preferred.

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in SEQ ID NO:X). Of course, a polynucleotide which hybridizes only to a poly A sequence (such as any 3' terminal poly(A) tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

Also encoded by nucleic acids of the invention are the amino acid sequences of the invention together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing. including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; and additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities.

Thus, the sequence encoding the polypeptide may be fused to a marker sequence, such as a sequence encoding a peptide which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz *et al., Proc. Natl. Acad. Sci. USA 86*:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson *et al., Cell 37*: 767 (1984). As discussed below, other such fusion proteins include those fused to Fc at the N- or C-terminus.

Sequences Encoding Signal Peptide and Secreted Portions

According to the signal hypothesis, proteins secreted by eukaryotic cells have a signal peptide (or secretory leader sequence) which is cleaved from the complete polypeptide to produce a secreted portion or "mature" form of the protein. Methods for predicting whether a protein has a signal peptide (or "secretory leader") as well as the cleavage point for that leader sequence are well known in the art. See, for instance, von Heinje, *supra*. The determined amino acid sequence of several proteins of the invention, determined by translation of the determined nucleotide sequence identified in Table 1, have been found to comprise an amino acid sequence of a secretory signal peptide. The sequence and cleavage site of that signal peptide are described in Table 1 and in the Examples and the signal sequence is underlined in the Figures, to the extent that these have been determined for each secreted protein of the invention.

More in particular, the present invention provides nucleic acid molecules encoding a secreted portion (mature form) of each secreted protein identified in Table 1. Most mammalian cells and even insect cells cleave signal peptides from secreted proteins with approximately the same specificity. However, in some cases, cleavage

of the signal peptide (as referred to herein as a "leader sequence" or "leader") from a secreted protein is not entirely uniform, which results in more than one secreted (also herein "mature") for or species of the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the initial polypeptide translated from its mRNA. Therefore, the present invention provides not only a determined nucleotide sequence and translated amino acid sequence identifying a signal peptide and secreted portion of each secreted protein of the invention, but also a deposited sample of a cDNA clone encoding a secreted (mature) form of each secreted protein of the invention.

More particularly, the invention further provides an isolated polypeptide comprising an amino acid sequence at least 90% identical, preferrably 95%, 96%, 97%, 98% or 99% identical, to a sequence of at least about 25, 50 or 100 contiguous amino acids in the complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for that cDNA clone in Table 1. A particularly preferred embodiment of this aspect of the invention is a polypeptide in which the sequence of contiguous amino acids is included in the amino acid sequence of a secreted portion of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. By the "secreted portion [or mature form] of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1" is meant the secreted portion(s) or mature form(s) of the protein produced by expression in any eukaryotic cell (for instance, cells of an established insect or mammalian cell line), preferably a human cell (for instance, cells of the well known HeLa cell line), of the complete open reading frame encoded by the human cDNA clone identified in Table 1 and contained in the deposit cited in Table 1.

Variant and Mutant Polynucleotides

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of the secreted proteins. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the secreted protein or portions thereof. Also especially preferred in this regard are conservative substitutions.

Most highly preferred are nucleic acid molecules encoding a secreted portion (mature form) of a protein described in Table 1 and having the amino acid sequence shown in the sequence listing as SEQ ID NO:X, or the amino acid sequence of the secreted portion (mature form) of the protein encoded by a deposited cDNA clone . Further embodiments include an isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 85% identical, more preferably at least 90% identical, and most preferably at least 95%, 96%, 97%, 98% or 99% identical to a polynucleotide of the invention described in Table 1, or a polynucleotide which hybridizes under stringent hybridization conditions to such a polynucleotide. This polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues. An additional nucleic acid embodiment of the invention relates to an isolated nucleic acid molecule comprising a polynucleotide

which encodes the amino acid sequence of an epitope-bearing portion of a secreted polypeptide having an amino acid sequence of SEQ ID NO:Y or an amino acid sequence of a secreted protein encoded by a cDNA clone in the deposit identified in Table 1.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a secreted polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the secreted polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in SEQ ID NO:1, or to the nucleotide sequence of a deposited cDNA can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2:482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such

that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

Uses for Nucleic Acid Molecules of the Invention

Each of the nucleic acid molecules identified herein can be used in numerous ways as polynucleotide reagents. The polynucleotides can be used as diagnostic probes for the presence of a specific mRNA in a particular cell type. In addition, these polynucleotides can be used as diagnostic probes suitable for use in genetic linkage analysis (polymorphisms) . Further, the polynucleotides can be used as probes for locating gene regions associated with genetic disease, as explained in more detail below.

The polynucleotides of the present invention are also valuable for chromosome identification. Each polynucleotide is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of cDNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in the sequence listing. Computer analysis of the sequences is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the secreted protein will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular nucleic acid sequence to a particular chromosome. Three or more clones can

be assigned per day using a single thermal cycler. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map a gene to its chromosome include in situ hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence in situ hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 500 or 600 bases; however, clones larger than 2,000 bp have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. For example, 2,000 bp is good, 4,000 is better, and more than 4,000 is probably not necessary to get good results a reasonable percentage of the time. For a review of this technique, see Verma et al., Human Chromosomes: a Manual of Basic Techniques. Pergamon Press, New York (1988).

Reagents for chromosome mapping can be used individually (to mark a single chromosome or a single site on that chromosome) or as panels of reagents (for marking multiple sites and/or multiple chromosomes). Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a polynucleotide sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library) .) The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb.)

Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that cDNA sequence. Ultimately, complete sequencing of genes from several individuals is required to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

In addition to the foregoing, the polynucleotides of the invention, as broadly described, can be used to control gene expression through triple helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide sequence to DNA or RNA. Polynucleotides suitable for use in these methods are usually 20 to 40 bases in length and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al, Nucl. Acids Res., 6:3073 (1979); Cooney et al, Science, 241:456 (1988) ; and Dervan et al, Science, 251: 1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem., 56:560 (1991) Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide.

Nucleic acid molecules of the present invention are also a useful in gene therapy which requires isolation of the disease-associated gene in question as a prerequisite to the insertion of a normal gene into an organism to correct a genetic defect. The high specificity of the cDNA probes according to this invention offer means of targeting such gene locations in a highly accurate manner.

The sequences of the present invention, as broadly defined, are also useful for identification of individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP.

However, RFLP is a pattern based technique, which does not require the DNA sequence of the individual to be sequenced. The polynucleotides and sequences of the present invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA. One can, for example, take a sequence of the invention and prepare two PCR primers. These are used to amplify an individual's DNA, corresponding to the gene or gene fragment. The amplified DNA is sequenced.

Panels of corresponding DNA sequences from individuals, made this way, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences, due to allelic differences. The sequences of the present invention can be used to particular advantage to obtain such identification sequences from individuals and from tissue, as further described in the Examples. The polynucleotide sequences shown in the sequence listing and the inserts contained in the deposited cDNAs uniquely represent portions of the human genome. Allelic

variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences comprising a part of the present invention can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals.

If a panel of reagents from sequences of this invention is used to generate a unique ID database for an individual, those same reagents can later be used to identify tissue from that individual. Positive identification of that individual, living or dead can be made from extremely small tissue samples.

Another use for DNA-based identification techniques is in forensic biology. PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, etc. In one prior art technique, gene sequences are amplified at specific loci known to contain a large number of allelic variations, for example the DQa class II HLA gene (Erlich, H., PCR Technology, Freeman and Co. (1992)). Once this specific area of the genome is amplified, it is digested with one or more restriction enzymes to yield an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene.

The sequences of the present invention can be used to provide polynucleotide reagents specifically targeted to additional loci in the human genome, and can enhance the reliability of DNA-based forensic identifications. Those sequences targeted to noncoding regions are particularly appropriate. As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Reagents for obtaining such sequence information are within the scope of the present invention. Such reagents can comprise complete genes, ESTs or corresponding coding regions, or

fragments of either of at least 20 bp, preferably at least 50 bp, most preferably at least 500 to 1,000 bp.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

The present application is directed to nucleic acid molecules at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence referenced in Table 1 and shown in the sequence listing or to the nucleic acid sequence of a deposited cDNA, irrespective of whether they encode a polypeptide having biological activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having biological activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, for one of the uses above.

Preferred, however, are nucleic acid molecules having sequences at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNA which do, in fact, encode a secreted polypeptide having biological activity. By "a polypeptide having biological activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the mature protein of the invention, as measured in a particular biological assay. "A polypeptide having biological activity" includes polypeptides that also exhibit any of the same activities as a protein of the invention in an assay in a dose-dependent manner. Although the degree of dose-dependent activity need not be identical to that of the protein, preferably, "a polypeptide having biological activity" will exhibit substantially similar dose-dependence in a given activity as compared to the protein (i.e., the candidate

polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity relative to the reference protein).

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of the deposited cDNA or the nucleic acid sequence shown in the sequence listing will encode a polypeptide "having biological activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having biological activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly affect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

Vectors, Host Cells and Protein Production

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and the production of polypeptides or fragments thereof by recombinant techniques. The vector may be, for example, a phage, plasmid, viral or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac*, *trp*, *phoA* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, Streptomyces and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293 and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc., *supra*; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods

are described in many standard laboratory manuals, such as Davis *et al.*, *Basic Methods In Molecular Biology* (1986).

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to stabilize and purify proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett *et al.*, *J. Molecular Recognition* 8:52-58 (1995) and K. Johanson *et al.*, *J. Biol. Chem.* 270:9459-9471 (1995).

A protein of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation,

acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention include: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

Polypeptides and Fragments

The invention further provides isolated polypeptides having an amino acid sequence encoded by a deposited cDNA, or an amino acid sequence in the sequence listing identified SEQ ID NO:Y as defined in Table 1, or a peptide or polypeptide comprising a portion of the above polypeptides. At the simplest level, the amino acid sequence can be synthesized using commercially available peptide synthesizers. This is particularly useful in producing small peptides and fragments of larger polypeptides. Such fragments are useful, for example, in generating antibodies against the native polypeptide.

Variant and Mutant Polypeptides

To improve or alter the characteristics of the polypeptides of the invention, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins" including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions.

For instance, for many proteins, including the mature form(s) of a secreted protein, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function. For instance, Ron et al., *J. Biol. Chem., 268*:2984-2988 (1993) reported modified KGF proteins that had heparin binding activity even if 3, 8, or 27 amino-terminal amino acid residues were missing. Similarly, many examples of biologically functional C-terminal deletion muteins are known. For instance, Interferon gamma shows up to ten times higher activities by deleting 8-10 amino acid residues from the carboxy terminus of the protein (Döbeli et al., *J. Biotechnology* 7:199-216 (1988). Furthermore, even if deletion of one or more amino acids from the N-terminus or C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature form of the protein generally will be retained when less than the majority of the residues of the complete or mature form of the protein are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

In addition to terminal deletion forms of the protein discussed above, it also will be recognized by one of ordinary skill in the art that some amino acid sequences

of a polypeptide can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity.

Thus, the invention further includes variants of a polypeptide which show substantial biological activity or which include regions of the protein such as the portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247*:1306-1310 (1990), wherein the authors indicate that there are two main approaches for studying the tolerance of an amino acid sequence to change. The first method relies on the process of evolution, in which mutations are either accepted or rejected by natural selection. The second approach uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene and selections or screens to identify sequences that maintain functionality.

As the authors state, these studies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at a certain position of the protein. For example, most buried amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Other such phenotypically silent substitutions are described in Bowie, J. U. *et al., supra*, and the references cited therein. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu. substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr.

Thus, the fragment, derivative or analog of a polypeptide shown in the figures (and sequence listing), or one encoded by the deposited cDNA, may be (i) one in

which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the above form of the polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the above form of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein

Thus, the mature polypeptide of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation. As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 2).

TABLE 2. CONSERVATIVE AMINO ACID SUBSTITUTIONS

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

Amino acids in the protein of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or *in vitro* or *in vitro* proliferative activity.

Of special interest are substitutions of charged amino acids with other charged or neutral amino acids which may produce proteins with highly desirable improved characteristics, such as less aggregation. Aggregation may not only reduce activity but also be problematic when preparing pharmaceutical formulations, because aggregates can be immunogenic (Pinckard *et al., Clin. Exp. Immunol.* 2:331-340 (1967); Robbins

et al., Diabetes 36: 838-845 (1987); Cleland *et al., Crit. Rev. Therapeutic Drug Carrier Systems 10*:307-377 (1993).

Replacement of amino acids can also change the selectivity of the binding of a ligand to cell surface receptors. For example, Ostade *et al., Nature 361*:266-268 (1993) describes certain mutations resulting in selective binding of TNF-α to only one of the two known types of TNF receptors. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al., J. Mol. Biol. 224*:899-904 (1992) and de Vos *et al. Science 255*:306-312 (1992)).

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide of the invention can be substantially purified by the one-step method described in Smith and Johnson, *Gene 67*:31-40 (1988). Polypeptides of the invention also can be purified from natural or recombinant sources using antibodies of the invention raised against the protein in methods which are well known in the art of protein purification.

Further polypeptides of the present invention include polypeptides which have at least 90% similarity, more preferably at least 95% similarity, and still more preferably at least 96%, 97%, 98% or 99% similarity to those described above. The polypeptides of the invention also comprise those which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to a polypeptide encoded by a deposited cDNA or to the polypeptide of SEQ ID NO:Y, and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

By "% similarity" for two polypeptides is intended a similarity score produced by comparing the amino acid sequences of the two polypeptides using the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison. WI 53711) and the default settings for determining similarity. Bestfit uses the local homology

algorithm of Smith and Waterman (Advances in Applied Mathematics 2:482-489, 1981) to find the best segment of similarity between two sequences.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a polypeptide described herein is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the polypeptide of the invention. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, an amino acid sequence shown in the sequence listing or to an amino acid sequence encoded by the deposited cDNA can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

The polypeptide of the present invention could be used as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art.

As described in detail below, the polypeptides of the present invention can also be used to raise polyclonal and monoclonal antibodies, which are useful in assays for detecting the corresponding protein expression as described below or as agonists and antagonists capable of enhancing or inhibiting function of the protein. Further, such polypeptides can be used in the yeast two-hybrid system to "capture" receptors of secreted proteins which are also candidate agonists and antagonists according to the present invention. The yeast two hybrid system is described in Fields and Song, Nature 340:245-246 (1989).

Epitope-Bearing Portions

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen *et al., Proc. Natl. Acad. Sci. USA 81*:3998- 4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R. A. (1983) "Antibodies that react with predetermined sites on proteins," *Science, 219*:660-666. Peptides capable of eliciting protein-reactive sera are

frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals. Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson *et al., Cell 37*:767-778 (1984) at 777.

Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. See, e.g., Houghten, R. A. (1985) "General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids." *Proc. Natl. Acad. Sci. USA 82*:5131-5135; this "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986).

Epitope-bearing peptides and polypeptides of the invention are used to induce antibodies according to methods well known in the art. See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow, M. *et al., Proc. Natl. Acad. Sci. USA 82*:910-914; and Bittle, F. J. *et al., J. Gen. Virol. 66*:2347-2354 (1985). Immunogenic epitope-bearing peptides of the invention, i.e., those parts of a protein that elicit an antibody response when the whole protein is the immunogen, are identified according to methods known in the art. See, for instance, Geysen et al., *supra*. Further still, U.S. Patent No. 5,194,392 to Geysen (1990) describes a general method of detecting or determining the sequence of monomers (amino acids or other compounds) which is a topological equivalent of the epitope (i.e., a "mimotope") which is complementary to a particular paratope (antigen binding site) of an antibody of interest. More

generally, U.S. Patent No. 4,433,092 to Geysen (1989) describes a method of detecting or determining a sequence of monomers which is a topographical equivalent of a ligand which is complementary to the ligand binding site of a particular receptor of interest. Similarly, U.S. Patent No. 5,480,971 to Houghten, R. A. et al. (1996) on Peralkylated Oligopeptide Mixtures discloses linear C1-C7-alkyl peralkylated oligopeptides and sets and libraries of such peptides, as well as methods for using such oligopeptide sets and libraries for determining the sequence of a peralkylated oligopeptide that preferentially binds to an acceptor molecule of interest. Thus, non-peptide analogs of the epitope-bearing peptides of the invention also can be made routinely by these methods.

Fusion Proteins

As one of skill in the art will appreciate, polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric secreted protein or protein fragment alone (Fountoulakis et *al., J. Biochem. 270*:3958-3964 (1995)).

Antibodies

Protein-species specific antibodies for use in the present invention can be raised against an intact protein or an antigenic polypeptide fragment thereof, which may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier.

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')2 fragments) which are capable of specifically binding to protein. Fab and F(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl *et al., J. Nucl. Med. 24*:316-325 (1983)). Thus, these fragments are preferred.

The antibodies of the present invention may be prepared by any of a variety of methods. For example, cells expressing the protein of the present invention or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of the secreted protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology (Köhler *et al., Nature 256*:495 (1975); Köhler *et al., Eur. J. Immunol. 6*:511 (1976); Köhler et al., Eur. J. Immunol. 6:292 (1976); Hammerling *et al., in: Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., (1981) pp. 563-681). In general, such procedures involve immunizing an animal (preferably a mouse) with a protein antigen of the invention or, more preferably, with a protein-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56° C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin. The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the American Type Culture Collection, Rockville, Maryland. After fusion, the resulting hybridoma cells

are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the protein antigen.

Alternatively, additional antibodies capable of binding to the protein antigen of the invention may be produced in a two-step procedure through the use of anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and that, therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the protein antigen. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce formation of further protein-specific antibodies.

It will be appreciated that Fab and F(ab')2 and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). Alternatively, protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For *in vivo* use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. See, for review, Morrison, *Science 229*:1202 (1985); Oi et al., *BioTechniques 4*:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO

8702671; Boulianne *et al., Nature 312*:643 (1984); Neuberger *et al., Nature 314*:268 (1985).

Identification and Diagnostic Applications

Assaying protein levels in a biological sample can occur using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., *et al., J. Cell. Biol. 101*:976-985 (1985); Jalkanen, M., *et al., J. Cell . Biol. 105*:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels. such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying protein levels in a biological sample obtained from an individual, protein can also be detected *in vivo* by imaging. Antibody labels or markers for *in vivo* imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I, ^{112}In, ^{99m}Tc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In

the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

Treatment of Conditions Related to Proteins of the Invention

It will be appreciated that conditions caused by a decrease in the standard or normal expression level of a protein of the invention, particularly a secreted protein, in an individual can be treated by administration of the polypeptide (in the form of a mature protein for secreted polypeptides). Thus, the invention also provides a method of treatment of an individual in need of an increased level of the protein of the present invention comprising administering to such an individual a pharmaceutical composition comprising an amount of the isolated polypeptide of the invention effective to increase the activity level of the protein in such an individual.

Formulations

Polypeptide composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the polypeptide alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of a polypeptide administered parenterally per dose will be in the range of about 1 µg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for

the hormone. If given continuously, the polypeptide is typically administered at a dose rate of about 1 µg/kg/hour to about 50 µg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing the protein of the invention may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

The polypeptide is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or mirocapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers 22*:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (R. Langer et al., *J. Biomed. Mater. Res. 15*:167-277 (1981), and R. Langer, *Chem. Tech. 12*:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposomally entrapped polypeptides. Liposomes containing the polypeptide are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. (USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the

lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal polypeptide therapy.

For parenteral administration, in one embodiment, the polypeptide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting the polypeptide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The polypeptide is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any polypeptide to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Polypeptides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized polypeptide using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Examples

EXAMPLE 1. Isolation of A Selected cDNA Clone From the Deposited Sample

Each protein of the invention is related to a human complementary DNA (cDNA) clone prepared from a messenger RNA (mRNA) encoding the related protein. The cDNA clone related to each protein of the invention is identified by a "cDNA Clone ID (Identifier)" in Table 1, below (e.g., "HABCE99"). DNA of each cDNA clone in Table 1 is contained in the material deposited with the American Type Culture Collection and given the ATCC Deposit Number shown for each cDNA Clone ID in Table 1. All deposits containing such clones have been submitted to the American Type Culture Collection (Rockville, Maryland USA) on the date indicated for each given accession number indicated in Table 1. All deposits have been made in accordance with the Budapest Treaty, and in full compliance with 37 CFR §1.801 et seq.

The cDNA clones contained in the ATCC deposits cited in Table 1 can be utilized by those of skill in the art by reference to the information describing each clone, and by reference to SEQ ID NO:X, provided in Table 1 for the determined nucleotide sequence of each deposited clone. The following additional information is provided for convenience. Each cDNA clone in a cited ATCC deposit is contained in a plasmid vector. Table 1 identifies the vector used to construct the cDNA library from which each clone was isolated. In many cases the vector used to construct the library is a phage vector from which a plasmid has been excised. The table immediately below provides a correlation of the related plasmid for each such phage vector used in construction of the cDNA library from which each cDNA clone listed in Table 1 originally was isolated. For example, where a particular clone is identified in Table 1 as being isolated in the vector "Lambda Zap," it can be seen from the following table that this cDNA clone contained in the biological deposit in pBluescript.

Vector Used to Construct Library	Corresponding DepositedPlasmid
Lambda Zap	pBluescript (pBS)
Uni-Zap XR	pBluescript (pBS)
Zap Express	pBK
lafmid BA	plafmid BA
pSport1	pSport1
pCMVSport 2.0	pCMVSport 2.0
pCMVSport 3.0	pCMVSport 3.0
pCR®2.1	pCR®2.1

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., *Nucleic Acids Res. 16:*7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., *Nucleic Acids Res. 17:*9494 (1989)) and pBK (Alting-Mees, M. A. et al., *Strategies 5:*58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both may be transformed into *E. coli* strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS-. The S and K refer to the orientation of the polylinker to the T7 and T3 primer sequences which flank the polylinker region ("S" is for *Sac*I and "K" is for *Kpn*I which are the first restriction enzyme sites on each respective end of the linker). "+" or "-" refer to the orientation of the f1 origin of replication ("ori"), such that in one orientation single stranded rescue initiated from the f1 ori generates sense strand DNA and in the other, antisense.

Vectors pSport1, pCMVSport 2.0 and pCMVSport 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, also available from Life Technologies. See, for instance, Gruber, C. E., et al., *Focus 15:*59- (1993). Vector lafmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into *E. coli* strain XL-1 Blue. Vector pCR®2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies. See, for instance, Clark, J. M., *Nuc. Acids Res. 16:*9677-9686 (1988) and Mead, D. *et al., Bio/Technology 9:* (1991).

The deposited material in the sample assigned the ATCC Deposit Number cited in Table 1 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, each cited deposit contains at least a plasmid for each cDNA clone identified in Table 1 as sharing the same ATCC Deposit Number.

Two approaches are used herein to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 1, although others are known in art. In the first, a plasmid is isolated directly by screening clones using an oligonucleotide probe. To isolate a particular clone, a specific oligonucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with ^{32}P-γ-ATP using T4 polynucleotide kinase and purified according to routine methods (e.g., Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY, 1982). The plasmid mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edit., (1989), Cold Spring Harbor

Laboratory Press, pages 1.93 to 1.104), or other technique known to those of skill in the art.

An alternative approach to isolate any polynucleotide of interest in the deposited library is to prepare two oligonucleotide primers of 17-20 nucleotides derived from both ends of the determined sequence for the selected clone (i.e., within the region of SEQ ID NO:X bounded by the 5' NT of the clone and the 3' NT of the clone defined in Table 1 for each cDNA clone identified therein. These two oligonucleotide primers are used to amplify the polynucleotide of interest using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 µl of reaction mixture with 0.5 ug of the above cDNA template. A convenient reaction mixture is 1.5-5 mM $MgCl_2$, 0.01% (w/v) gelatin, 20 µM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not limited to filter probing, clone enrichment using specific probes and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res., 21(7):1683-1684 (1993). Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcript and a primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest, is

used to PCR amplify the 5' portion of the desired full-length gene which may then be sequenced and used to generate the full length gene. This method starts with total RNA isolated from the desired source; poly A RNA may be used but is not a prerequisite for this procedure. The RNA preparation may then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RN-A ligase step. The phosphatase if used is then inactivated and the RNA is treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase. This modified RNA preparation can then be used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis-reaction can then be used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

EXAMPLE 2. Features of Proteins of the Invention

Table 1, below, describes particular features of the proteins and related nucleotide and amino acid sequences of this invention.

TABLE 1. FEATURES OF PROTEINS OF THE INVENTION

Protein ID (Group-Nr)	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO:X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	NOTE	5' NT of Start Codon	5' NT of First AA	AA SEQ ID NO:Y	First AA	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
PF353-1	HEMFI85	209053 05/16/97	pBluescript SK-	1	1091	1	1091		118	118	2	1			103
PF353-2	HTXET53	209053 05/16/97	pBluescript SK-	3	887	1	887		64	64	4	1	15	16	172
PF353-3	HT3SG28	209053 05/16/97	pBluescript SK-	5	540	1	540		19	19	6	1	22	23	88
PF353-4	HBZAK03	209053 05/16/97	pSport 1.0	7	520	1	520		112	112	8	1			59
PF353-5	HDFUB43	209053 05/16/97	pBluescript SK-	9	1352	1	1352		55	55	10	1			116
PF353-6	HEBGM49	209054 05/16/97	pBluescript SK-	11	632	1	632		88	88	12	1			150
PF353-7	HNGBH54	209054 05/16/97	Uni-ZAP XR	13	582	1	582		1	1	14	1			193
PF353-8	HSAAL25	209054 05/16/97	pBluescript SK-	15	1356	1	1356		115	115	16	1			324
PF353-9	HUSAX55	209054 05/16/97	pBluescript SK-	17	2934	1	2934		1	1	18	1			977
PF353-10	HSXCK41	209054 05/16/97	pBluescript SK-	19	1587	1	1587		1	1	20	1	15	16	528
PF353-11	HFKFY79	209054 05/16/97	pBluescript SK-	21	1359	1	1359		1	1	22	1			452
PF353-12	HAICH28	209054 05/16/97	Uni-Zap XR	23	1098	1	1098		1	1	24	1			365

FEATURES OF THE PROTEIN ENCODED BY SEQ ID NO: 1

The novel full-length chemotactic cytokine V (CCV) polypeptide exhibits significant sequence identity to a chemotactic protein isolated from the murine S100 fraction designated CP-10 (chemotactic protein, 10 kD). The chemotactic cytokine V cDNA clone contains an 1091 nucleotide insert (SEQ ID NO:1) which encodes a 103 amino acid polypeptide (SEQ ID NO:2), both shown in Figure 1. The clone was obtained from an induced endothelial cell cDNA library. A sequence alignment analysis of the deduced amino acid sequence of HEMFI85 shows that CCV shares approximately 24% identity and 69% similarity to the amino acid sequence of the murine CP-10 protein. In addition, it was determined by a BLAST analysis that the amino acid sequence of chemotactic cytokine V also exhibits approximately 31% identity and 67% similarity to the previously described rat intracellular Ca2+-binding protein. An examination of expression of chemotactic cytokine V in the HGS database reveals a widespread cell and tissue distribution of this gene. Expression of this clone was observed in a wide variety of human cDNA libraries in the Human Genome Sciences, Inc. (HGS) express sequence tag (EST) database including colon carcinoma (HCC) cell line, smooth muscle, amygdala depression, keratinocytes, uninduced endothelial cells, osteoblasts, and others.

CP-10 is a potent factor capable of extravascular recruitment of polymorphonuclear cells (PMN) and monocytes from circulation. Optimal chemotactic activity of CP-10 for murine PMN and neutrophils is in the range of 10-11 and 10-13 M, making this factor one of the most potent chemotactic factors reported to date. CP-10 is the murine homologue of a human S100 protein designated migration inhibition factor-related protein 8 (MRP8). MRP 8 can occur as a complex with an additional human S100 protein termed MRP14 (the complex has previously been reported as the cystic fibrosis antigen, calgranulin A and B, or L1 antigen). This complex can comprise as much as 10-20% of the total cytoplasmic protein content of resting neutrophils and, although a significantly lower percentage of total cytoplasmic protein content, MRP8/14 complexes can also be found in resting monocytes. There

is also evidence that suggests that MRP8/14 may be released from myeloid cells, although it is not clear whether the complex is actively released as part of a response to inflammation or passively as a part of the demise of such cells during the inflammatory process.

The function(s) of MRP8/14 complexes, CP-10, and related S100 fraction Ca2+-binding proteins are not entirely clear. However, it is thought that a major functional role of such proteins is in the recruitment of certain populations of immune cells to areas of inflammation. Devery and coworkers (J. Immunol. 152, 1888-1897; 1994) have demonstrated that expression of cell surface molecules such as Mac-1, which is involved in the process of cell adhesion as well as several additional cellular processes, may be influenced by prior interaction of the cell with chemotactic factors such as CP-10. These studies have also been performed in vivo where it was observed that CP-10 protein accumulated on the endothelial lining of small blood vessels in LPS-inflamed footpads. Furthermore, increased levels of MRP8/14 have been observed in the sera of patients afflicted with several inflammatory diseases including rheumatoid arthritis. It has also been suggested that chemotactic cytokine molecules such as CP-10 or MRP8/14 may function as a type of "calcium sink" during times of elevated intracellular levels of calcium for sustained periods of time. Alternatively, it has been suggested that MRP8/14 may function as a specific inhibitor of casein kinase II acitivity. Although the precise functional role(s) of many of the currently defined chemotactic cytokine-like proteins containing significant regions of sequence identity to HEMFI85 are not known in any detail, a number of studies with these proteins strongly suggest one or more roles for these proteins in a variety of human disease states including rheumatoid arthritis, sarcoidosis, tuberculosis, onchocerciasis, and other chronic inflammatory disease states. As a result, the discovery of a novel chemotactic cytokine-like molecule is believed to be of value in a variety therapeutic and diagnostic capacities.

Owing to the homology to CP-10 and other calcium binding proteins it is expected that the CCV polypeptide shares possess common bioactivities. The

activity of CCV may be assayed by any of several biological assays known in the art, preferrably calcium binding assays. The homology to CP-10 and other calcium binding proteins indicates that the CCV polypeptide is useful in the detection and treatment of chronic inflammatory diseases such as rheumatoid arthritis, sarcoidosis, tuberculosis and onchocerciasis.

FEATURES OF THE PROTEINS ENCODED BY SEQ ID NOS: 3 and 5

The full-length nucleotide sequences of two novel human cDNA clones (HTXET53 and HT3SG28) which encode splice variants of the previously reported and highly related chemokines LAG-2, NKG5, and 519 have recently been identified. See for example, Hercend and Triebel (WPI Acc. No. 90-132241/17). These two clones have been designated Chemokine from Activated T-Cells-1 (CAT-1) (HTXET53), and Chemokine from Activated T-Cells-2 (CAT-2) (HT3SG28).

The HTXET53 clone was obtained from a human activated (12 hour) T-cell cDNA library and contains a 887 nucleotide insert (SEQ ID NO:3) which encodes a 172 amino acid polypeptide (SEQ ID NO:4), shown in Figure 2. The HT3SG28 clone was obtained from a human activated (8 hour) T-cell cDNA library and contains a 550 nucleotide insert (SEQ ID NO:5) which encodes an 88 amino acid polypeptide (SEQ ID NO:6), shown in Figure 3. The predicted amino acid sequences of the novel full-length CAT splice variants contain several regions of nearly perfect sequence identity to the previously reported human LAG-2, NKG5, and 519 lymphokines. Alignment of the amino acid sequences shows perfect identity between the two novel molecules with LAG-2 and NKG5, with the exception of a 27 amino acid insertion near the amino terminus of HTXET53, and a 57 amino acid deletion very near the carboxy terminus of HT3SG28. The 519 amino acid sequence differs from each of the novel clones and from LAG-2 and NKG5 by an 18 amino acid deletion of the hydrophobic amino terminus.

The HTXET53 polypeptide is predicted to have a 15 amino acid secretory leader sequence. The HT3SG28 polypeptide is predicted by the computer program

PSORT to have either a 15 or a 22 amino acid leader sequence. The leader sequences are underlined in Figures 2 and 3. Applicants believe that both the shorter and longer form of the HT3SG28 polypeptides (i.e., begining at either residue 16 or residue 23) are active.

Expression profiles of the two novel genes are qualitatively identical in the HGS database. Additional HGS human cDNA libraries which contain the two novel CAT clones are resting T-cells, apoptotic T-cells, activated T-cells, spleen (chronic lymphocytic leukemia), activated monocytes, pituitary, and 9 week early stage human. The mRNA expression patterns of these novel genes have not been examined by Northern blot analysis.

The original molecule cloned from this group the T-cell-specific clone 519. NKG5 was a term used to describe a group of identical clones isolated from a human natural killer (NK) cell cDNA library. These genes are highly related and are thought to be expressed only in NK and T-cells. A genomic clone of the gene which encodes both 519 and NKG5 consists of at least five exons and four introns which are likely responsible for the generation of the related, but unique gene products. The genomic clone also reveals a number of T-cell-specific and activation state-specific regulatory sequences indicating that expession of the gene is highly restricted to certain functions of a small subset of cell types.

The novel and previously described molecules discussed herein also contain approximately 33% identity with a recently reported clone designated NK-lysin. NK-lysin has been found to exhibit a potent anti-bacterial activity against such organisms as Escherichia coli, Bacillus megaterium, Acinetobacter calcoaceticus, and Streptococcus pyogenes. In addition, NK-lysin was also observed to possess a marked lytic activity against an NK-cell-sensitive mouse tumor cell line (YAC-1), but had no such activity against erythrocytes. As a result, there are a number of potential therapeutic and/or diagnostic applications for a factor such as those encoded by HTXET53 and HT3SG28. Applications may include the detection and treatment of such clinical presentations as various bacterial infections, a number of lymphomas,

immunological disorders, autoimmune diseases, inflammatory diseases, various allergies, and possibly as anti-infectious agents.

FEATURES OF THE PROTEINS ENCODED BY SEQ ID NOS: 7 and 9

The novel Melanoma Inhibitory Activity Protein (MIA)-2 and -3 cDNA clones presented herein are shown in Figures 4 and 5. The cDNA clone HBZAK03 contains a 520 nucleotide insert (SEQ ID NO:7) which encodes a 59 amino acid polypeptide (SEQ ID NO:8), as shown in Figure 4. A BLAST analysis of the predicted amino acid sequence of HBZAK03 demonstrates that this novel clone appears to be a splice variant of another cDNA clone designated HLFBD44. The nucleotide sequence of HLFBD44 (SEQ ID NO:9) and deduced amino acid sequence (SEQ ID NO:10) are shown in Figure 5. Both of these HGS clones exhibit significant sequence identity to a human gene termed melanoma inhibitory activity (MIA) protein. BestFit analysis demonstrates that the HBZAK03 protein exhibits approximately 20% identity and 58% similarity to the MIA protein over a region of roughly 60 amino acids. The expression profile of the HBZAK03 cDNA in the HGS database reveals that it appears in a number of HGS human cDNA libraries in addition to the prostate cDNA library from which it was cloned. Some of the cDNA libraries in which this clone appears include fetal lung, the bone marrow cell line (RS4;11), macrophage, serum-treated smooth muscle, epileptic frontal cortex, subtracted fetal brain, HSA 172 cell line, induced endothelial cells, and others.

The highest sequence identity of the novel cDNA clones presented herein suggests that they may possess a function involved in the regulation of melanoma progression. The previously described MIA protein functions as a component of a highly complex and only partially characterized system of stimulatory and inhibitory factors which together dictate the progression of a melanoma. MIA is secreted by malignant melanoma cells and has the capacity to inhibit the growth of melanoma cells in culture. Investigators have examined the expression profile of the MIA gene by Northern blot and RT-PCR analysis and have determined that it is expressed in all

melanoma cell lines, a few glioma cell lines, approximately half of the benign melanomas, all malignant melanomas, and from all lymph node metastases of malignant melanomas examined (Bosserhoff et al., J. Biol Chem. 271, 490-495; 1996). In contrast, no MIA expression was detected by these methods in samples obtained from any other skin-derived cells including normal fibroblasts, HaCaT keratinocytes, COS cells, HeLa cells, HepG2 cells, DU 145 (human prostate carcinoma) cells, and J82 (human bladder carcinoma) cells.

Based on the sequence similarity between these polypeptides MIA-2 and -3 are predicted to be useful in the detection and regulation of malignant melanoma, in immune system modulation, and in the treatment of cardiac arrest and stroke. Other activities of MIA-1 as well as assays for detecting MIA-1 activity are outlined in WO 95/03328, hereby incorporated herein by reference in its entirety. MIA-2 and -3 activity can be assayed accordingly.

FEATURES OF THE PROTEINS ENCODED BY SEQ ID NOS: 11 and 13

A macrophage-specific protein, termed AIF-1, has only very recently been molecularly cloned. AIF-1 appears to function in macrophage activation in the pathogenesis of chronic cardiac rejection following transplantation. A characteristic manifestation of cardiac tissue rejection following transplantation is an immune-mediated arteriosclerosis which ultimately results in graft failure and creates the need for retransplantation during the first postoperative year. It is thought that the arteriosclerotic state results from an alloimmune response involving activated immune cells, particularly macrophages, which stimulate smooth muscle-cell migration and proliferation into the area of the transplant leading to lesions in donor vessels. AIF-1 was identified by Utans and coworkers (J. Clin. Invest. 95, 2954-2962; 1995) in ongoing studies of inducible gene expression patterns in macrophage cells in a chronic rejecting rat heart allograft model. AIF-1 was expressed in response to INF-g in the chronic cardiac rejection model referenced above. Expression of AIF-1 was seen

selectively in activated macrophages, neutrophils, and the macrophage-like cell lines THP-1, U937, and HL60, but not in several other human cells and tissues examined. Furthermore, low levels of AIF-1 expression can be observed in endomyocardial biopsy samples obtained from human heart transplant patients.

The cDNA clone designated HEBGM49 or "AIF-2" contains a 632 nucleotide cDNA insert (SEQ ID NO:11) encoding a 150 amino acid polypeptide (SEQ ID NO:12), as shown in Figure 6. The cDNA clone was isolated from a human early stage brain cDNA library. This clone also appears in several other cDNA libraries constructed from a variety of human cell and tissue types including fetal epithelium, fetal kidney, hippocampus, tongue, and osteoblastoma HOS cells. A BLAST analysis of the amino acid sequence of HEBGM49 demonstrated that this clone exhibits approximately 65% identity and 80% similarity with AIF-1 over its entire length.

The cDNA clone HNGBH45 or "AIF-3" contains a 757 nucleotide cDNA insert (SEQ ID NO:13) encoding a 193 amino acid polypeptide (SEQ ID NO:14), as shown in Figure 7. The cDNA clone was isolated from a human neutrophil cDNA library. This clone appears in a number of additional cDNA libraries including aortic endothelium, cerebellum, corpus collosum, CD34-depleted buffy coat, activated neutrophil, colon cancer, resting T-cells, tonsils, and others. A BLAST analysis of the amino acid sequence of HNGBH45 demonstrated that this clone exhibits approximately 25% identity and 47% similarity over approximately 70 amino acids of the AIF-1 molecule.

AIF-2 and AIF-3 are believed to be valuable clinical markers for assessing varying degrees of acute and chronic rejection of transplanted cardiac tissue. In addition, monitoring the level of AIF-2 and/or AIF-3 expression may also be useful in determining the level of macrophage or neutrophil infiltration into area of the transplanted tissue. In addition, AIF-2 and -3 may be used as targets in assays for the identification of antagonists such as small orgainic molecules which act to block AIF activity. Such assays are known in the art.

FEATURES OF PROTEIN ENCODED BY SEQ ID NO: 15

The full-length nucleotide sequence of a novel human cDNA clone (HSAAL25) has been isolated which is believed to encode a new member of the annexin/lipocortin supergene family. The novel polypeptide is termed herein "Annexin HSAAL25". The annexin/lipocortin supergene family is composed of at least ten calcium-binding proteins proposed to function in a variety of cellular roles including phospholipase A2 and protein kinase C inhibition, anti-coagulation, endo- and exo-cytosis, inositol phosphate metabolism, and as calcium channel proteins. Eukaryotic calcium-binding proteins are typically classified as proteins which bind calcium by a mechanism which either includes or does not include an E-F hand motif. The annexin/lipocortin superfamily is the largest group of calcium-binding proteins whose interaction with calcium is not mediated by an E-F hand motif. Structurally, all known annexins may be characterized by a common carboxy terminal region consisting of four similar amino acid sequences, of approximately seventy amino acids each, termed the "annexin repeats". Conversely, the amino termini of annexin/lipocortin proteins vary widely in both length and amino acid composition between member protein sequences. Typical expression patterns of annexin/lipocortin proteins include a wide variety of cells and tissues including lung, kidney, bone marrow, spleen, thymus, brain, macrophage, placenta, ovary, uterus, skeletal muscle, and others.

Annexin/lipocortin proteins are involved in a wide variety of physiologically important cellular processes. For example, lipocortin-1 (LC-1; also known as annexin-I) appears to function as a second messenger in the anti-inflammatory glucocorticoid signal transduction cascade. Most LC-1 molecules are cell surface-associated and attached to the plasma membrane by a Ca2+-dependent interaction with unrelated plasma membrane binding molecules. The process of extravasation, in which polymorphonuclear leukocytes (PMNs) migrate into an area of inflammation, adhere to the vascular wall, and eventually pass through the vascular wall into the surrounding tissue, may be delayed by glucocorticoids, and, as a result of LC-1

function, the overall process of inflammation may be delayed. As an example of the diversity of LC-1, and other annexin/lipocortin superfamily member, function, LC-1 has also been shown to play a major regulatory role in a number of possibly unrelated cellular systems such as cell growth regulation and differentiation, response of the CNS to cytokines, neuroendocrine secretion, anti-coagulation, and neurodegeneration.

Annexin HSAAL25 contains a 1356 nucleotide cDNA insert (SEQ ID NO:15) encoding a 324 amino acid polypeptide (SEQ ID NO:16), as is shown in Figure 8. HSAAL25 was isolated from a cDNA library made from the HSA 172 cell line. Although previously described annexin/lipocortin proteins are widely expressed, this clone also appears only once in the HSA 172 cell line cDNA library and does not appear in any other tissue type assayed for. A BLAST analysis of the amino acid sequence of HSAAL25 demonstrated that this clone exhibits at least 30% identity and 55% similarity over the entire length of a molecule designated human annexin-III, a member of the annexin/lipocortin supergene family.

There is clearly a need for identifying and exploiting novel members of the annexin/lipocortin superfamily such as the cDNA clone described herein. Plasma membrane-associated molecules, such as the novel potential members of the annexin/lipocortin superfamily detailed here, should prove useful in target based screens for small molecules and other such pharmacologically valuable factors that may be useful for regulating the complex processes of inflammation. Furthermore, Annexin HSAAL25 is believed to be useful as a regulator of coagulation (anti-coagulant) by affecting Ca2+-dependent cell to cell aggregation. In addition, this annexin-like clone may prove valuable in a number of other therapeutically useful roles as an anti-inflammatory agent including regulation of ischemia, tumor metastasis, rheumatoid arthritis, other inflammatory diseases, wound healing, arteriosclerosis, and other heart diseases.

FEATURES OF PROTEIN ENCODED BY SEQ ID NO: 17

The full-length nucleotide sequence of a novel human cDNA (HUSAX55) which encodes a previously unidentified "ES/130-like I" protein has been identified. The translation product of the novel full-length ES/130-like I cDNA clone exhibits significant sequence identity to the chicken EDTA-soluble/130 kDa protein (ES/130) gene. The ES/130-like I cDNA clone contains an 3036 nucleotide insert (SEQ ID NO:17) which encodes a 977 amino acid polypeptide (SEQ ID NO:18), as shown in Figure 9. The clone was obtained from an umbilical vein endothelial cell cDNA library. A BLAST analysis of the deduced amino acid sequence of HUSAX55 exhibits approximately 66% identity and 83% similarity to the amino acid sequence of the chicken ES/130 gene over a 573 amino acid stretch. Expression of ES/130-like I is detected in a wide collection of HGS human cDNA libraries including amygdala depression, thymus, smooth muscle, endometrial tumor, synovial sarcoma, macrophage, fetal heart, and a number of others. Northern blot analyses performed on expression of the ES/130-like I gene indicates a high level of expression in pancreas and liver and moderate to low expression elsewhere.

The in vitro process of endothelial cell transformation to mesenchymal tissue models a similar in vivo process in the developing heart where closely associated epithelial cells undergo a transformation to cardiac mesenchyme tissue. This transformation is a required event for the development of a multichambered heart from the primative, single chambered heart tube. ES/130 was originally identified as a 130 kD antigen isolated from the 100,000 x g pellet fraction of non-cytolytic EDTA extracts of developing chicken cardiac tissue. Inclusion of this fraction in cardiac endothelial cell cultures results in formation of mesenchymal tissue. ES/130 is an extracellular, secreted protein which, in addition to endothelial cell transformation, has been proposed to function in the regulation of adhesion molecule expression and limb bud ectoderm, neural tube, and notocord development. Potential therapeutic and/or diagnostic applications for the ES130-like I protein include such clinical presentations as atherosclerosis, restenosis, or as a general factor following a number of types of surgery.

FEATURES OF THE PROTEIN ENCODED BY SEQ ID NO: 19

The full-length nucleotide sequence of a human cDNA clone (HSXCK41) which encodes a novel brain-enriched hyaluronan-binding factor ("BEF") has been determined. The novel BEF cDNA clone presented herein was discovered in a human substantia nigra cDNA library. The clone contains a 1757 nucleotide insert (SEQ ID NO:19) which is predicted to encode a 528 amino acid polypeptide (SEQ ID NO:20). A BLAST analysis of the predicted amino acid sequenc of HSXCK41 demonstrates significant sequence identity to the bovine brevican mRNA (GenBank entry X75887), a member of the aggrecan/versican family of cell surface proteoglycans. The HSXCK41 amino acid sequence exhibits approximately 92% identity and 95% similarity over an approximately 400 amino acid stretch of the brevican sequence. This clone has been identified in a number additional HGS human cDNA libraries, many of which originate from neural tissues. These include epileptic frontal cortex, early stage brain, skin tumor, hippocampus, cerebellum, hemangiopericytoma, infant brain, fetal brain, and fetal bone.

The aggrecan/versican family of cell surface proteoglycans may be characterized by the presence of chondroitin sulfate side chains, a hyaluronic acid (HA)-binding motif in the amino terminal domain, and at least one epidermal growth factor (EGF)-like repeat, a lectin-like motif, and one or more complement regulatory protein (CRP)-like motifs in the carboxy terminal domain. The aggrecan/versican family includes a number of members such as brevican, aggrecan, decorin, versican, and neurocan. Brevican is expressed predominantly in the brain and in primary cerebellar astrocytes, but not in neurons. Meanwhile, both aggrecan and versican are expressed in chondrocytes in human articular cartilage obtained from subjects of a wide range of ages. Aggrecan messenger RNAs undergo alternative splicing events which vary the inclusion or exclusion of the single EGF-like motif in the carboxy terminal domain. Alternatively, versican contains two EGF-like motifs and a single CRP-like motif, all of which are present in all expression patterns examined. Finally, the expression of

two recently described members of the aggrecan/versican family isolated from the human sciatic nerve is significantly increased following lesioning of the nerve.

The functional roles of members of the aggrecan/versican family are rather varied. Aggrecan itself aggregates with HA to function as a major space-filling component of cartilage. Brevican, an aggrecan/versican family member which is a conditional chondroitan sulfate proteoglycan, appears in a secreted, soluble form as well as in a GPI-anchored form. Both brevican isoforms have been implicated as functional components of the terminally differentiating and adult nervous systems. It will likely be determined that molecules such as these and the novel BEF cDNA clone HSXCK41 may play a role in one or more of a variety of cellular processes which typically involve intercellular contact and communication mediated through cell surface and/or secreted glycoprotein factors. Such cellular processes might include cell adhesion, proliferation, tumor metastasis, and lymphocyte migration into areas of inflammation. Related polypeptides are believed to be expressed at a higher level in tumors such as gliomas. Thus, BEF polynucleotides and polypeptides are useful as diagnostic markers and reagents for detection of tumors such as gliomas.

FEATURES OF THE PROTEIN ENCODED BY SEQ ID NO: 21

The full-length nucleotide sequence of a human cDNA clone (HFKFY79) which encodes a novel adipose differentiation factor ("ADF") has recently been determined. The novel ADF cDNA clone presented herein was originally isolated from a human fetal kidney cDNA library. The clone contains a 1550 nucleotide insert (SEQ ID NO:21) which encodes a 452 amino acid polypeptide (SEQ ID NO:22), as shown in Figure 11. A BLAST analysis of the predicted amino acid sequence of HFKFY79 demonstrates that this clone exhibits its highest degree of sequence relatedness in the GenBank public database to the murine ADF protein (GenBank accession number M93275). Based on its homology to murine ADF, human ADF is believed to share common biological activities. A BestFit analysis of the predicted amino acid sequence of HFKFY79 versus the murine ADF amino acid sequence demonstrates that the two

protein sequences exhibit approximately 39% identity and 79% similarity. The expression profile of the HFKFY79 clone suggests a widely distributed expression pattern. In addition to the human fetal kidney library from which this clone was obtained, it also appears in a large number of human cDNA libraries including ulcerative colitis, adult testis, hypothalamus, induced endothelial cells, Jurkat T-cell line in S-phase, serum-treated and control smooth muscle, adipocytes, adult small intestine, lymph node breast cancer, infant brain, and many others.

The murine ADF gene was cloned by Jiang & Serrero (Proc. Natl. Acad. Sci. USA 89, 7856-7860; 1992, incorporated herein by reference) in an effort to identify genes whose expression profiles change significantly during the process of 1246 adipocyte cell and primary adipocyte differentiation. The murine ADF gene product identified by Jiang & Serrero is a 50 kD, membrane-bound protein expressed abundantly in mouse fat pads. The novel cDNA presented herein also exhibits sequence identity to several additional lipid-specific proteins. The first of the putative homologues is the major substrate for cAMP-dependent protein kinase A (PKA) in adipocytes and is termed perilipin. Perilipin is expressed in two alternatively spliced forms designated perilipins A and B. Both forms of perilipins are expressed exclusively at the surface of lipid storage droplets. It is thought that perilipids may function as a barrier to deny access of lipase to lipid reservoir of unstimulated cells. This event may be regulated by PKA-dependent phosphorylation of perilipin which allows exposure of lipid molecules to lipase. In addition, ADF is also related by sequence identity to a gene cloned from a human bone marrow-derived stromal cell line (KM-102) designated adipogenesis inhibitory factor (AGIF). AGIF has been shown to inhibit the process of adipogenesis in the mouse preadipocyte cell line 3T3-L1. Thus, human ADF may be useful among other things as a therapeutic modulator of lipid metabolism in the human body.

FEATURES OF THE PROTEIN ENCODED BY SEQ ID NO: 23

The novel "Bcl-like" cDNA clone (HAICH28) presented herein was originally identified in a TNF-a/IFN-induced endothelial cell cDNA library. The clone contains a 1211 nucleotide insert (SEQ ID NO:23) which encodes a 365 amino acid polypeptide (SEQ ID NO:24). A BLAST analysis of the predicted amino acid sequence of HAICH28 demonstrates that this clone exhibits strong sequence similarity to two previously reported genes termed bovine polyA binding protein II and human Bcl-w (GenBank accession numbers X89969 and U59747, respectively). The expression profile of the HAICH28 clone suggests a widely distributed expression pattern. In addition to the TNF-a/IFN-induced endothelial cell library from which this clone was obtained, it also appears in a large number of human cDNA libraries including PHA-stimulated T-cells, osteoblasts, schizophrenic hypothalamus, activated monocytes, adrenal gland tumor, primary dendritic cells, and a number of others.

The protein product of the related Bcl-w gene has been determined to function as a key player in the cellular apoptosis or cell death pathway. Apoptosis is a term which describes the process of programmed cell death in vertebrates. During the process of apoptosis, the cell membrane shrinks and blebs resulting in a loss of membrane integrity and intercellular contact. In addition, the chromatin is condensed and cleaved into a characteristic ladder-like organization and, finally, vesicular remnants of the cell are quickly engulfed and destroyed by neighboring cells. The signal for the cell to enter the apoptotic pathway likely begins with the binding of Fas ligand or tumor necrosis factor (TNF), or the recently discovered TRAIL ligand. to the Fas/CD95/APO-1 or TNF (p55), or DR4 or DR5 receptors, respectively. These ligand/receptor interactions recruit a cellular protein designated FLICE to the cell membrane to act as a physical link between the Fas/CD95/APO-1 and TNF receptor complexes, also termed death receptors, and the cysteine proteases belonging to the

interleukin-1b (IL-1b) converting enzyme (ICE)/CED-3 family to carry out the process of apoptosis.

The t(14:18) chromosomal translocation is often associated with human follicular lymphoma. In this chromosomal abnormality, the immunoglobulin heavy chain locus becomes translocated adjacent to the Bcl-2 gene, resulting in a drastic overexpression of the Bcl-2 gene. Bcl-2 blocks the process of apoptosis by an unknown mechanism. It has been proposed that Bcl-2 controls the process of apoptosis by regulating endoplasmic reticulum-associated Ca2+ fluxes. Several other genes have been identified which have significant regions of sequence identity with Bcl-2, including Ced-9, BHRF1, Bax, Bcl-xS, Bcl-xL, Bcl-w, Bak, Mcl-1, and GRS. The protein product of each of these genes can affect the process of apoptosis in either a positive (for example, Bax or Bcl-xS) or negative (for example Bcl-2, BHRF1, Ced-9, or Bcl-xL) fashion.

A large number of cells fall victim to the apoptotic process throughout development and during the lifetime of the organism. Clearly, strict regulation of the functional molecules comprising such a potentially dangerous process is an extremely necessary and valuable facet of the repertoire of cellular regulatory pathways. As a result, the identification of novel molecules related to Bcl-2 or Bcl-w, such as that encoded by the novel cDNA clone described herein, represents a major step in understanding, and, in turn, exploiting the complex process of controlled cell death. Accordingly, the Bcl-like polypeptide of the present invention is thought to be useful as a therapeutic in an anti-viral or anti-tumor capacity or, alternatively, in a diagnostic capacity.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 53, line N/A.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution
American Type Culture Collection

Address of depositary institution *(including postal code and country)*
12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
May 16, 1997	209053

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

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A. The indications made below relate to the microorganism referred to in the description on page 53, line N/A.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution
American Type Culture Collection

Address of depositary institution *(including postal code and country)*
12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
May 16, 1997	209054

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

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What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide selected from the group consisting of:

(a) the polypeptide shown in SEQ ID NO:2;

(b) the polypeptide shown in SEQ ID NO:4;

(c) the mature polypeptide shown as residues 16-172 in SEQ ID NO:4;

(d) the polypeptide shown in SEQ ID NO:6;

(e) the mature polypeptide shown as residues 16-88 in SEQ ID NO:6;

(f) the mature polypeptide shown as residues 23-88 in SEQ ID NO:6;

(g) the polypeptide shown in SEQ ID NO:8;

(h) the polypeptide shown in SEQ ID NO:10;

(i) the polypeptide shown in SEQ ID NO:12;

(j) the polypeptide shown in SEQ ID NO:14;

(k) the polypeptide shown in SEQ ID NO:16;

(l) the polypeptide shown in SEQ ID NO:18;

(m) the polypeptide shown in SEQ ID NO:20;

(n) the mature polypeptide shown as residues 16-528 in SEQ ID NO:20;

(o) the polypeptide shown in SEQ ID NO:22; and

(p) the polypeptide shown in SEQ ID NO:24.

2. The nucleic acid molecule of claim 1 comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, and SEQ ID NO:23.

3. An isolated nucleic acid molecule of claim 3 comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

4. An isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule of claim 1, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

5. An isolated nucleic acid molecule of claim 6 comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

6. An isolated polypeptide comprising an amino acid sequence which is identical to a sequence of at least about 10 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1.

7. An isolated polypeptide of claim 6 comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y.

8. An isolated polypeptide comprising an amino acid sequence identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

9. A method of making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 1 into a vector.

10. A recombinant vector produced by the method of claim 9.

11. A method of making a recombinant host cell comprising introducing a vector of claim 10 into a host cell.

12. A recombinant host cell produced by the method of claim 12.

13. A method of making an isolated polypeptide comprising culturing a recombinant host cell of claim 12 under conditions such that said polypeptide is expressed and recovering said polypeptide.

14. An isolated polypeptide produced by the method of claim 13.

15. An isolated antibody capable of specifically binding to a polypeptide of claim 6.

HEMFI85 (SEQ ID NOS:1 and 2)

```
                10        20        30        40        50        60
                .         .         .         .         .         .
  1 AGC CCG GCT GGG CTG AGC GCA GGG AGC TGC TTG GCA GTG CCA GAG CCC AGG CCC CAG AGC 60

                70        80        90       100       110       120
                .         .         .         .         .         .
 61 CCT GCT GGA GAG GAG GCA GAC TGA GGC AGC AGG CCC CGC CAG CAG GCG AAC AGG GAG ATG 120
  1                                                                 M  1

               130       140       150       160       170       180
                .         .         .         .         .         .
121 TCA GAC TGC TAC ACG GAG CTG GAG AAG GCA GTC ATT GTC CTG GTG GAA AAC TTC TAC AAA 180
  2  S  D  C  Y  T  E  L  E  K  A  V  I  V  L  V  E  N  F  Y  K  21

               190       200       210       220       230       240
                .         .         .         .         .         .
181 TAT GTG TCT AAG TAC AGC CTG GTC AAG AAC AAG ATC AGC AAG AGC AGC TTC CGC GAG ATG 240
 22  Y  V  S  K  Y  S  L  V  K  N  K  I  S  K  S  S  F  R  E  M  41

               250       260       270       280       290       300
                .         .         .         .         .         .
241 CTC CAG AAA GAG CTG AAC CAC ATG CTG TCG GAC ACA GGG AAC CGG AAG CTG CGG ATT AAG 300
 42  L  Q  K  E  L  N  H  M  L  S  D  T  G  N  R  K  L  R  I  K  61
```

FIG. 1A

```
                310         320         330         340         350         360
                .           .           .           .           .           .
301 CTC ATC CAG AAC CTG GAT GCC AAT CAT GAT GGG CGC ATC AGC TTC GAT GAG TAC TGG ACC 360
 62  L   I   Q   N   L   D   A   N   H   D   G   R   I   S   F   D   E   Y   W   T  81

                370         380         390         400         410         420
                .           .           .           .           .           .
361 TTG ATA GGC GGC ATC ACC GGC CCC ATC GCC AAA CTC ATC CAT GAG CAG GAG CAG CAG AGC 420
 82  L   I   G   G   I   T   G   P   I   A   K   L   I   H   E   Q   E   Q   Q   S  101

                430         440         450         460         470         480
                .           .           .           .           .           .
421 AGC AGC TAG AGA CCC CTT TGG CCA CAC CTT CCA GGC ACT GGC CTG ATG CCC CGC CCT GGT 480
102  S   S   *                                                                    103

                490         500         510         520         530         540
                .           .           .           .           .           .
481 GCT CTC CCC AGG CTC CCT CCT CAG CCT CCT GCC CAC CCA GGG CCC TTT ACT CTC TTC TCC 540

                550         560         570         580         590         600
                .           .           .           .           .           .
541 CTC CAG ACC TTC CTC TGA CCC TTG CTG AAC TGG GGT CCC TTT GTG AGT GTC TCA GTC TAG 600

                610         620         630         640         650         660
                .           .           .           .           .           .
601 AGG TAC CTC CCT CCC TGG GGG GTC TCA GCT CCT GGA GTC GCA GGC CCT TGG GGC CCC TCT 660
```

FIG. 1B


```
                670         680         690         700         710         720
                .           .           .           .           .           .
661 GTG AGA TCT CAA TGC TGT CTG GGG ACC CTA AGA GTT TTC TCA CCT GTT CAG TCT CAT CTA 720

                730         740         750         760         770         780
                .           .           .           .           .           .
721 ACC TTC CAA TGT CTG ATG TTC CTG CCA AAT TCC TGC CTG ATT CTG GGT CCG TCC TGA CCT 780

                790         800         810         820         830         840
                .           .           .           .           .           .
781 CCA AAG GTC AGC TTG GTG CTT GAG GTC TCC CTG CTC TTG GTG GCA GTG GTA GCA GCA ACA 840

                850         860         870         880         890         900
                .           .           .           .           .           .
841 GCA GCA GCA GCA GCA GCA GCA GCA GCA GAG ACC TCT CCA CTT TCC CTT AGC CCC TCT GCT 900

                910         920         930         940         950         960
                .           .           .           .           .           .
901 GGG TAG AGA GGC ACT TTC AGG GAC TTC CCT CCA GCT GCC TCT TCA TCT GGG AAT GAG CTA 960

                970         980         990        1000        1010        1020
                .           .           .           .           .           .
961 AGC AAG GCT GAG CCT CCT CCT GTT GCT TGA AAT AAT GAT GAT ATA AAG GCT GGA TTT GGA 1020
```

FIG. 1C

```
                1030          1040          1050          1060          1070          1080
                 .             .             .             .             .             .
1021 GTT TGT ATC CCC TGG TCC TCT GGG ATG CTC ATT AAA ACC TTC CCA CTC CTT GAA AAA AAA 1080

                1090
                 .
1081 AAA AAA AAA AA 1091
```

FIG. 1D

HTXET53 (SEQ ID NOS:3 and 4)

```
          10                  30                  50
GCACGAGGCAGGCTCCCTGCCCATAAAACAGGGTGTGAAAGGCATCTCAGCGGCTGCCCC
          70                  90                  110
ACCATGGCTACCTGGGCCCTCCTGCTCCTTGCAGCCATGCTCCTGGGCAACCCAGGCCTT
   M  A  T  W  A  L  L  L  L  A  A  M  L  L  G  N  P  G  L
         130                 150                 170
GAGGTCAGTGTGAGCCCCAAGGGCAAGAACACTTCTGGAAGGGAGAGTGGATTTGGCTGG
E  V  S  V  S  P  K  G  K  N  T  S  G  R  E  S  G  F  G  W
         190                 210                 230
GCCATCTGGATGGAAGGTCTGGTCTTCTCTCGTCTGAGCCCTGAGTACTACGACCTGGCA
A  I  W  M  E  G  L  V  F  S  R  L  S  P  E  Y  Y  D  L  A
         250                 270                 290
AGAGCCCACCTGCGTGATGAGGAGAAATCCTGCCCGTGCCTGGCCCAGGAGGGCCCCCAG
R  A  H  L  R  D  E  E  K  S  C  P  C  L  A  Q  E  G  P  Q
         310                 330                 350
GGTGACCTGTTGACCAAAACACAGGAGCTGGGCCGTGACTACAGGACCTGTCTGACGATA
G  D  L  L  T  K  T  Q  E  L  G  R  D  Y  R  T  C  L  T  I
```

FIG. 2A

```
        430                 450                 470
ACCCGGGTGTGTAGGACGGGGAGGTCACGATGGCGCGACGTCTGCAGAAATTTCATGAGG
T  R  V  C  R  T  G  R  S  R  W  R  D  V  C  R  N  F  M  R
        490                 510                 530
AGGTATCAGTCTAGAGTTACCCAGGGCCTCGTGGCCGGAGAAACTGCCCAGCAGATCTGT
R  Y  Q  S  R  V  T  Q  G  L  V  A  G  E  T  A  Q  Q  I  C
        550                 570                 590
GAGGACCTCAGGTTGTGTATACCTTCTACAGGTCCCCTCTGAGCCCTCTCACCTTGTCCT
E  D  L  R  L  C  I  P  S  T  G  P  L  *
        610                 630                 650
GTGGAAGAAGCACAGGCTCCTGTCCTCAGATCCCGGGAACCTCAGCAACCTCTGCCGGCT
        670                 690                 710
CCTCGCTTCCTCGATCCAGAATCCACTCTCCAGTCTCCCTCCCCTGACTCCCTCTGCTGT
        730                 750                 770
CCTCCCCTCTCACGAGAATAAAGTGTCAAGCAAGATTTTAGCCGCAGCTGCTTCTTCTTT
        790                 810                 830
GGTGGATTTGAGGGGTGGGTGTCAGTGGCATGCTGGGGTGAGCTGTGTAGTCCTTCAATA
        850                 870
AATGTCTGTCGTGTGTCCCATAAAAAAAAAAAAAAAAAAAAAAAAA
```

FIG. 2B

T3SG28 (SEQ ID NOS:5 and 6)

```
          10                  30                  50
CTCAGCGGCTGCCCCACCATGGCTACCTGGGCCCTCCTGCTCCTTGCAGCCATGCTCCTG
                    M  A  T  W  A  L  L  L  L  A  A  M  L  L
          70                  90                  110
GGCAACCCAGGTCTGGTCTTCTCTCGTCTGAGCCCTGAGTACTACGACCTGGCAAGAGCC
G  N  P  G  L  V  F  S  R  L  S  P  E  Y  Y  D  L  A  R  A
          130                 150                 170
CACCTGCGTGATGAGGAGAAATCCTGCCCGTGCCTGGCCCAGGAGGGCCCCCAGGGTGAC
H  L  R  D  E  E  K  S  C  P  C  L  A  Q  E  G  P  Q  G  D
          190                 210                 230
CTGTTGACCAAAACACAGGAGCTGGGCCGTGACTACAGGACCTGTCTGACGATAGTCCAA
L  L  T  K  T  Q  E  L  G  R  D  Y  R  T  C  L  T  I  V  Q
          250                 270                 290
AAACTGAAGAAGATGGTGGATAAGCCCACCCCAGGTCCCCTCTGAGCCCTCTCACCTTGT
K  L  K  K  M  V  D  K  P  T  P  G  P  L  *
```

FIG. 3A

```
        310                 330                 350
CCTGTGGAAGAAGCACAGGCTCCTGTCCTCAGATCCCGGGAACCTCAGCAACCTCTGCCG
        370                 390                 410
GCTCCTCGCTTCCTCGATCCAGAATCCACTCTCCAGTCTCCCTCCCCTGACTCCCTCTGC
        430                 450                 470
TGTCCTCCCCTCTCACGAGAATAAAGTGTCAAGCCAGAAAAAAAAAAAAAAAAAAAAAA
        490                 510                 530
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
        550
AAAAAAAAAA
```

FIG. 3B

HBZAK03 (SEQ ID NOS:7 and 8)

```
          10                  30                  50
CGACCCACGCGTCCGGTTCGCTCTCTGGTAAGGGCGTGCAGGTGTTGGCCGCGGCCTCTG
          70                  90                 110
AGCTGGGATGAGCCGTGCTCCCGGTGGAAGCAAGGGAGCCCAGCCGGAGCCATGGCCAGT
                                                   M   A   S
        130                 150                 170
ACAGTGGTAGCAGTTGGACTGACCATTGCTGCTGCAGGATTTGCAGGCCGTTACGTTTTG
T   V   V   A   V   G   L   T   I   A   A   A   G   F   A   G   R   Y   V   L
        190                 210                 230
CAAGCCATGAAGCATATGGAGCCTCAAGTAAAACAAGTTTTTCAAAGCCTACCAAAATCT
Q   A   M   K   H   M   E   P   Q   V   K   Q   V   F   Q   S   L   P   K   S
        250                 270                 290
GCCTTCAGTGGTGGCTATTATAGAGCCCTACTGCCAATAAAGGGAAAATAAGAGATGCTC
A   F   S   G   G   Y   Y   R   A   L   L   P   I   K   G   K   *
        310                 330                 350
ATCGACGAATTATGCTTTTAAATCATCCTGACAAAGGAGGATCTCCTTATATAGCAGCCA
        370                 390                 410
AAATCAATGAAGCTAAAGATTTACTAGAAGGTCAAGCTAAAAAATGAAGTAAATGTATGA
        430                 450                 470
TGAATTTTAAGTTCGTATTAGTTTATGTATATGAGTACTAAGTTTTTATAATAAAATGCT
        490                 510
CCAGAGCTACAATTTTAACAAACAATTAAAAAAAAAAAAA
```

FIG. 4

HLFBD44 (SEQ ID NOS:9 and 10)

```
          10                  30                  50
CTGAGCTGGGATGAGCCGTGCTCCCGGTGGAAGCAAGGGAGCCCAGCCGGAGCCATGGCC
                                                    M  A
          70                  90                  110
AGTACAGTGGTAGCAGTTGGACTGACCATTGCTGCTGCAGGATTTGCAGGCCGTTACGTT
S  T  V  V  A  V  G  L  T  I  A  A  A  G  F  A  G  R  Y  V
         130                 150                 170
TTGCAAGCCATGAAGCATATGGAGCCTCAAGTAAAACAAGTTTTTCAAAGCCTACCAAAA
L  Q  A  M  K  H  M  E  P  Q  V  K  Q  V  F  Q  S  L  P  K
         190                 210                 230
TCTGCCTTCAGTGGTGGCTATTATAGAGGTGGGTTTGAACCCAAAATGACAAAACGGGAA
S  A  F  S  G  G  G  Y  Y  R  G  G  F  E  P  K  M  T  K  R  E
         250                 270                 290
GCAGCATTAATACTAGGTGTAAGCCCTACTGCCAATAAAGGGAAAATAAGAGATGCTCAT
A  A  L  I  L  G  V  S  P  T  A  N  K  G  K  I  R  D  A  H
         310                 330                 350
CGACGAATTATGCTTTTAAATCATCCTGACAAAGGAGGATCTCCTTATATAGCAGCCAAA
R  R  I  M  L  L  N  H  P  D  K  G  G  S  P  Y  I  A  A  K
         370                 390                 410
ATCAATGAAGCTAAAGATTTACTAGAAGGTCAAGCTAAAAAATGAAGTAAATGTATGATG
I  N  E  A  K  D  L  L  E  G  Q  A  K  K  *
```

FIG.5A


```
         430                 450                 470
AATTTTAAGTTCGTATTAGTTTATGTATATGAGTACTAAGTTTTTATAATAAAATGCCTC
         490                 510                 530
AGAGCTACAATTTTAAAAAATGATTTAGCACAAGCTAAATCTCAAAGCCTTGGTATAATT
         550                 570                 590
TTCTTGTTTAAATTTGGGGATTTTAAATCAGATTATAGTTTAGAATATTTGCGTATTAAT
         610                 630                 650
TATGGGCAAGCACACACCTTCTGAATAGAAATATTGTTCATTACTCATTTAGCAGATAAT
         670                 690                 710
TTGGGACCTATGTCTACTTTTCAAGGCAAAGTGAAGATGACAGTCCTTGCTCTCAGGGAG
         730                 750                 770
CCCCCACTTTAATGGGAGACTGATAAACTGGTAATTAGACTGTGATAAATAGTATGATGG
         790                 810                 830
AAATTAGCTTAAGCTGTTTAAGTAGGGACTCTTCTTATTCGGTGGAAAGGCTGTTCCAGG
         850                 870                 890
TACAGGCAACTGGCCTGGCAACTTGGATACTTGGAACCTTGTATTTAAAAGTGAATTTAA
         910                 930                 950
CCACAACTGAGACCTAAGAAATTGACCTAGGGGTGTGTGTGTGTGTGTATTCTATGTACA
         970                 990                1010
TATAAACCCATTTTTATTTCATGCATTAAAAATAGTATGATAAAGATTTCAGAGTACAGG
        1030                1050                1070
TCTGGTACAATCACAGTTCATTGCAGCCTCAACCTCCTGGGTTTAAGCAGTCCTCCCGCC
        1090                1110                1130
TCAGCCTCCCAAAGTACTGGGATTACAGGCATGAGTATTTACATTGTATTCAGCTAGCCC
```

FIG.5B

```
      1150                1170                1190
CTTAAAGGTAATGACCATTTATAAATTATTCCTTCAGTTGGCTATTTCTTGACATAATCA
      1210                1230                1250
AACTTCTGCAATTGTTATGATTAAGCTTAAACCCTGTTAGCAAAACTGAAAACTGAAATG
      1270                1290                1310
TTCTCAATATCAACATATTTAATTTGGACTCTTTAGAATTTATACACTAATAAATTTAAA
      1330                1350
TGATGTTTAAAGGCAAAAAAAAAAAAAAAAAA
```

FIG.5C

HEBGM49 (SEQ ID NOS:11 and 12)

```
          10                  30                  50
CACGAGGCCCGGACCAGGCGCCTGTGCCTCCTCCTCGTCCCTCGCCGCGTCCGCGAACCT
          70                  90                  110
GGAGCCGGCGGGAGCCCCGCGCTCGCCATGTCGGGCGAGCTCAGCAACAGGTTCCAAGGA
                            M   S   G   E   L   S   N   R   F   Q   G
        130                 150                 170
GGGAAGGCGTTCGGCTTGCTCAAAGCCCGGCAGGAGAGGAGGCTGGCCGAGATCAACCGG
G   K   A   F   G   L   L   K   A   R   Q   E   R   R   L   A   E   I   N   R
        190                 210                 230
GAGTTTCTGTGTGACCAGAAGTACAGTGATGAAGAGAACCTTCCAGAAAAGCTCACAGCC
E   F   L   C   D   Q   K   Y   S   D   E   E   N   L   P   E   K   L   T   A
        250                 270                 290
TTCAAAGAGAAGTACATGGAGTTTGACCTGAACAATGAAGGCGAGATTGACCTGATGTCT
F   K   E   K   Y   M   E   F   D   L   N   N   E   G   E   I   D   L   M   S
        310                 330                 350
TTAAAGAGGATGATGGAGAAGCTTGGTGTCCCCAAGACCCACCTGGAGATGAAGAAGATG
L   K   R   M   M   E   K   L   G   V   P   K   T   H   L   E   M   K   K   M
        370                 390                 410
ATCTCAGAGGTGACAGGAGGGGTCAGTGACACTATATCCTACCGAGACTTTGTGAACATG
I   S   E   V   T   G   G   V   S   D   T   I   S   Y   R   D   F   V   N   M
```

FIG.6A

```
        430                 450                 470
ATGCTGGGGAAACGGTCGGCTGTCCTCAAGTTAGTCATGATGTTTGAAGGAAAAGCCAAC
M  L  G  K  R  S  A  V  L  K  L  V  M  M  F  E  G  K  A  N
        490                 510                 530
GAGAGCAGCCCCAAGCCAGTTGGCCCCCCTCCAGAGAGAGACATTGCTAGCCTGCCCTGA
E  S  S  P  K  P  V  G  P  P  P  E  R  D  I  A  S  L  P  *
        550                 570                 590
GGACCCCGCCTGGACTCCCCAGCCTTCCCACCCCATACCTCCCTCCCGATCTTGCTGCCC
        610                 630
TTCTTGACACACTGTGATCCGGCACGAGCGGC
```

FIG. 6B

HNGBH54 (SEQ ID NOS:13 and 14)

```
                10          20          30          40          50          60
                .           .           .           .           .           .
1 ATG GGC AGC GCG GAC TGC GAG CTG AGC GCC AAG CTG CTG CGG CGC GCA GAC CTC AAC CAG 60
1 M   G   S   A   D   C   E   L   S   A   K   L   L   R   R   A   D   L   N   Q  20

                70          80          90          100         110         120
                .           .           .           .           .           .
61 GGC ATC GGC GAG CCC CAG TCG CCC AGC CGC CGC GTC TTC AAC CCC TAC ACC GAG TTC AAG 120
21 G   I   G   E   P   Q   S   P   S   R   R   V   F   N   P   Y   T   E   F   K  40

                130         140         150         160         170         180
                .           .           .           .           .           .
121 GAG TTC TCC AGG AAG CAG ATC AAG GAC ATG GAG AAG ATG TTC AAG CAG TAT GAT GCC GGG 180
 41 E   F   S   R   K   Q   I   K   D   M   E   K   M   F   K   Q   Y   D   A   G  60

                190         200         210         220         230         240
                .           .           .           .           .           .
181 CGG GAC GGC TTC ATC GAC CTG ATG GAG CTA AAA CTC ATG ATG GAG AAA CTT GGG GCC CCT 240
 61 R   D   G   F   I   D   L   M   E   L   K   L   M   M   E   K   L   G   A   P  80

                250         260         270         280         290         300
                .           .           .           .           .           .
241 CAG ACC CAC CTG GGC CTG AAA AAC ATG ATC AAG GAG GTG GAT GAG GAC TTT GAC AGC AAG 300
 81 Q   T   H   L   G   L   K   N   M   I   K   E   V   D   E   D   F   D   S   K 100
```

FIG. 7A

```
                310         320         330         340         350         360
                .           .           .           .           .           .
301 CTG AGC TTC CGG GAG TTC CTC CTG ATC TTC CGC AAG GCG GCG GCC GGG GAG CTT CAG GAG 360
101  L   S   F   R   E   F   L   L   I   F   R   K   A   A   A   G   E   L   Q   E 120

                370         380         390         400         410         420
                .           .           .           .           .           .
361 GAC AGC GGG CTG TGC GTG CTG GCC CGC CTC TCT GAG ATC GAC GTC TCC AGT GAG GGT GTC 420
121  D   S   G   L   C   V   L   A   R   L   S   E   I   D   V   S   S   E   G   V 140

                430         440         450         460         470         480
                .           .           .           .           .           .
421 AAG GGG GCC AAG AGC TTC TTT GAG GCC AAG GTC CAG GCC ATC AAC GTG TCC AGC CGC TTC 480
141  K   G   A   K   S   F   F   E   A   K   V   Q   A   I   N   V   S   S   R   F 160

                490         500         510         520         530         540
                .           .           .           .           .           .
481 GAG GAG GAG ATC AAG GCA GAG CAG GAG GAA AGG AAG AAG CAG GCG GAG GAG ATG AAG CAG 540
161  E   E   E   I   K   A   E   Q   E   E   R   K   K   Q   A   E   E   M   K   Q 180

                550         560         570         580
                .           .           .           .
541 CGG AAA GCG GCC TTC AAG GAG CTG CAG TCC ACC TTT AAG TAG 582
181  R   K   A   A   F   K   E   L   Q   S   T   F   K   * 193
```

FIG. 7B

HSAAL25 (SEQ ID NOS:15 and 16)

```
          10                  30                  50
ACATATTTACATTTGATTTAACAGTGAACCTTAATTCTTTCTGGCTTCACAGTGAAACAA
          70                  90                  110
GTTTATGCAATCGATCAAATATTTTCATCCCTGAGGTTAACAATTACCATCAAAATGTTT
                                                    M   F
         130                 150                 170
TGTGGAGACTATGTGCAAGGAACCATCTTCCCAGCTCCCAATTTCAATCCCATAATGGAT
C   G   D   Y   V   Q   G   T   I   F   P   A   P   N   F   N   P   I   M   D
         190                 210                 230
GCCCAAATGCTAGGAGGAGCACTCCAAGGATTTGACTGTGACAAAGACATGCTGATCAAC
A   Q   M   L   G   G   A   L   Q   G   F   D   C   D   K   D   M   L   I   N
         250                 270                 290
ATTCTGACTCAGCGCTGCAATGCACAAAGGATGATGATTGCAGAGGCATACCAGAGCATG
I   L   T   Q   R   C   N   A   Q   R   M   M   I   A   E   A   Y   Q   S   M
         310                 330                 350
TATGGCCGGGACCTGATTGGGGATCTGAGGGAGCAGCTTTCGGATCACTTCAAAGATGTG
Y   G   R   D   L   I   G   D   L   R   E   Q   L   S   D   H   F   K   D   V
         370                 390                 410
ATGGCTGGCCTCATGTACCCACCACCACTGTATGATGCTCATGAGCTCTGGCATGCCATG
M   A   G   L   M   Y   P   P   P   L   Y   D   A   H   E   L   W   H   A   M
```

FIG. 8A

```
        430                 450                 470
AAGGGAGTAGGCACTGATGAGAATTGCCTCATTGAAATACTAGCTTCAAGAACAAATGGA
K  G  V  G  T  D  E  N  C  L  I  E  I  L  A  S  R  T  N  G
        490                 510                 530
GAAATTTTCCAGATGCGAGAAGCCTACTGCTTGCAATACAGCAATAACCTCCAAGAGGAC
E  I  F  Q  M  R  E  A  Y  C  L  Q  Y  S  N  N  L  Q  E  D
        550                 570                 590
ATTTATTCAGAGACCTCGGGACACTTCAGAGATACTCTCATGAACTTGGTCCAGGGGACC
I  Y  S  E  T  S  G  H  F  R  D  T  L  M  N  L  V  Q  G  T
        610                 630                 650
AGAGAGGAAGGATATACAGACCCTGCGATGGCTGCTCAGGATGCAATGGTCCTATGGGAA
R  E  E  G  Y  T  D  P  A  M  A  A  Q  D  A  M  V  L  W  E
        670                 690                 710
GCCTGTCAGCAGAAGACGGGGGAGCACAAAACCATGCTGCAAATGATCCTGTGCAACAAG
A  C  Q  Q  K  T  G  E  H  K  T  M  L  Q  M  I  L  C  N  K
        730                 750                 770
AGCTACCAGCAGCTGCGGCTGGTTTTCCAGGAATTTCAAAATATTTCTGGGCAAGATATG
S  Y  Q  Q  L  R  L  V  F  Q  E  F  Q  N  I  S  G  Q  D  M
        790                 810                 830
GTAGATGCCATTAATGAATGTTATGATGGATACTTTCAGGAGCTGCTGGTTGCAATTGTT
V  D  A  I  N  E  C  Y  D  G  Y  F  Q  E  L  L  V  A  I  V
        850                 870                 890
CTCTGTGTTCGAGACAAACCAGCCTATTTTGCTTATAGATTATATAGTGCAATTCATGAC
L  C  V  R  D  K  P  A  Y  F  A  Y  R  L  Y  S  A  I  H  D
```

FIG.8B


```
        910                 930                 950
TTTGGTTTCCATAATAAAACTGTAATCAGGATTCTCATTGCCAGAAGTGAAATAGACCTG
F  G  F  H  N  K  T  V  I  R  I  L  I  A  R  S  E  I  D  L
        970                 990                1010
CTGACCATAAGGAAACGATACAAAGAGCGATATGGAAAATCCCTATTTCATGATATCAGA
L  T  I  R  K  R  Y  K  E  R  Y  G  K  S  L  F  H  D  I  R
       1030                1050                1070
AATTTTGCTTCAGGGCATTATAAGAAAGCACTGCTTGCCATCTGTGCTGGTGATGCTGAG
N  F  A  S  G  H  Y  K  K  A  L  L  A  I  C  A  G  D  A  E
       1090                1110                1130
GACTACTAAAATGAAGAGGACTTGGAGTACTGTGCACTCCTCTTTCTAGACACTTCCAAA
D  Y  *
       1150                1170                1190
TAGAGATTTTCTCACAAATTTGTACTGTTCATGGCACTATTAACAAAACTATACAATCAT
       1210                1230                1250
ATTTTCTCTTCTATCTTTGAAATTATTCTAAGCCAAAGAAAACTATGAATGAAAGTATAT
       1270                1290                1310
GATACTGAATTTGCCTACTATCCTGAATTTGCCTACTATCTAATCAGCAATTAAATAAAT
       1330                1350
TGTGCATGATGGAATAATAAAAAAAAAAAAAAAAA
```

FIG.8C

HUSAX55 (SEQ ID NOS:17 and 18)

```
                10        20        30        40        50        60
                .         .         .         .         .         .
  1 ATGGATATTTACGACACTCAAACCTTGGGGGTTGTGGTCTTTGGAGGATTCATGGTTGTT 60
  1  M  D  I  Y  D  T  Q  T  L  G  V  V  V  F  G  G  F  M  V  V  20

                70        80        90        100       110       120
                .         .         .         .         .         .
 61 TCTGCCATTGGCATCTTCCTGGTGTCGACTTTCTCCATGAAGGAAACGTCATATGAAGAA 120
 21  S  A  I  G  I  F  L  V  S  T  F  S  M  K  E  T  S  Y  E  E  40

                130       140       150       160       170       180
                .         .         .         .         .         .
121 GCCCTAGCCAACCAGCGCAAGGAGATGGCGAAAACTCACCACCAGAAAGTCGAGAAGAAA 180
 41  A  L  A  N  Q  R  K  E  M  A  K  T  H  H  Q  K  V  E  K  K  60

                190       200       210       220       230       240
                .         .         .         .         .         .
181 AAGAAGGAGAAAACAGTGGAGAAGAAAGGAAAGACCAAGAAAAAGGAAGAGAAACCTAAT 240
 61  K  K  E  K  T  V  E  K  K  G  K  T  K  K  K  E  E  K  P  N  80
```

FIG.9A


```
                250       260       270       280       290       300
                .         .         .         .         .         .
241 GGGAAGATACCTGATCATGATCCAGCCCCCAATGTGACTGTCCTCCTTCGAGAACCAGTG 300
 81  G  K  I  P  D  H  D  P  A  P  N  V  T  V  L  L  R  E  P  V  100

                310       320       330       340       350       360
                .         .         .         .         .         .
301 CGGGCTCCTGCTGTGGCTGTGGCTCCAACCCCAGTGCAGCCCCCCATTATCGTTGCTCCT 360
101  R  A  P  A  V  A  V  A  P  T  P  V  Q  P  P  I  I  V  A  P  120

                370       380       390       400       410       420
                .         .         .         .         .         .
361 GTCGCCACAGTTCCAGCCATGCCCCAGGAGAAGCTGGCCTCCTCCCCCAAGGACAAAAAG 420
121  V  A  T  V  P  A  M  P  Q  E  K  L  A  S  S  P  K  D  K  K  140

                430       440       450       460       470       480
                .         .         .         .         .         .
421 AAGAAGGAGAAAAAAGTGGCAAAAGTGGAACCAGCTGTCAGCTCTGTAGTGAATTCCATC 480
141  K  K  E  K  K  V  A  K  V  E  P  A  V  S  S  V  V  N  S  I  160
```

FIG.9B

```
            490         500         510         520         530         540
              .           .           .           .           .           .
481 CAGGTTCTCACTTCGAAGGCTGCCATCTTGGAAACTGCTCCCAAGGAGGGCAGAAATACA 540
161  Q  V  L  T  S  K  A  A  I  L  E  T  A  P  K  E  G  R  N  T  180

            550         560         570         580         590         600
              .           .           .           .           .           .
541 GATGTGGCCCAGAGCCCAGAGGCACCAAAGCAAGAGGCTCCTGCCAAGAAGAAGTCTGGT 600
181  D  V  A  Q  S  P  E  A  P  K  Q  E  A  P  A  K  K  K  S  G  200

            610         620         630         640         650         660
              .           .           .           .           .           .
601 TCAAAGAAAAAAGGGCCCCCAGATGCCGACGGCCCTCTCTACCTCCCCTACAAGACGCTG 660
201  S  K  K  K  G  P  P  D  A  D  G  P  L  Y  L  P  Y  K  T  L  220

            670         680         690         700         710         720
              .           .           .           .           .           .
661 GTCTCCACGGTTGGGAGCATGGTGTTCAACGAGGGCGAGGCCCAGCGGCTCATCGAGATC 720
221  V  S  T  V  G  S  M  V  F  N  E  G  E  A  Q  R  L  I  E  I  240
```

FIG.9C


```
            730         740         750         760         770         780
              .           .           .           .           .           .
721 CTGTCTGAGAAGGCTGGCATCATTCAGGACACCTGGCACAAGGCCACTCAGAAGGGTGAC 780
241  L  S  E  K  A  G  I  I  Q  D  T  W  H  K  A  T  Q  K  G  D  260

            790         800         810         820         830         840
              .           .           .           .           .           .
781 CCTGTGGCGATTCTGAAACGCCAGCTGGAAGAGAAGGAAAAACTGCTGGCCACAGAACAG 840
261  P  V  A  I  L  K  R  Q  L  E  E  K  E  K  L  L  A  T  E  Q  280

            850         860         870         880         890         900
              .           .           .           .           .           .
841 GAAGATGCGGCTGTCGCCAAGAGCAAACTGAGGGAGCTCAACAAGGAGATGGCAGCAGAA 900
281  E  D  A  A  V  A  K  S  K  L  R  E  L  N  K  E  M  A  A  E  300

            910         920         930         940         950         960
              .           .           .           .           .           .
901 AAGGCCAAAGCAGCAGCCGGGGAGGCCAAAGTGAAAAAGCAGCTGGTGGCCCGGGAGCAG 960
301  K  A  K  A  A  A  G  E  A  K  V  K  K  Q  L  V  A  R  E  Q  320
```

FIG.9D

```
            970        980        990       1000       1010       1020
             .          .          .          .          .          .
961 GAGATCACGGCTGTGCAGGCACGCATGCAGGCCAGCTACCGGGAGCACGTGAAGGAGGTG 1020
321  E  I  T  A  V  Q  A  R  M  Q  A  S  Y  R  E  H  V  K  E  V  340

           1030       1040       1050       1060       1070       1080
             .          .          .          .          .          .
1021 CAGCAGCTGCAGGGCAAGATCCGGACTCTTCAGGAGCAGCTGGAGAATGGCCCCAACACG 1080
341  Q  Q  L  Q  G  K  I  R  T  L  Q  E  Q  L  E  N  G  P  N  T  360

           1090       1100       1110       1120       1130       1140
             .          .          .          .          .          .
1081 CAGCTGGCCCGCCTGCAGCAGGAGAACTCCATCCTGCGGGATGCCTTGAACCAGGCCACG 1140
361  Q  L  A  R  L  Q  Q  E  N  S  I  L  R  D  A  L  N  Q  A  T  380

           1150       1160       1170       1180       1190       1200
             .          .          .          .          .          .
1141 AGCCAGGTGGAGAGCAAGCAGAACGCAGAGCTGGCCAAGCTTCGGCAGGAGCTCAGCAAG 1200
381  S  Q  V  E  S  K  Q  N  A  E  L  A  K  L  R  Q  E  L  S  K  400

           1210       1220       1230       1240       1250       1260
             .          .          .          .          .          .
1201 GTCAGCAAAGAGCTGGTGGAGAAGTCAGAGGCTGTGCGGCAAGATGAGCAGCAGCGGAAA 1260
401  V  S  K  E  L  V  E  K  S  E  A  V  R  Q  D  E  Q  Q  R  K  420
```

FIG. 9E


```
           1270       1280       1290       1300       1310       1320
             .          .          .          .          .          .
1261 GCTCTGGAAGCCAAGGCAGCTGCCTTCGAGAAGCAGGTCCTGCAGCTGCAGGCGTCCCAC 1320
421  A  L  E  A  K  A  A  A  F  E  K  Q  V  L  Q  L  Q  A  S  H  440

           1330       1340       1350       1360       1370       1380
             .          .          .          .          .          .
1321 AGGGAGAGTGAGGAGGCCCTGCAGAAGCGCCTGGACGAGGTCAGCCGGGAGCTGTGCCAC 1380
441  R  E  S  E  E  A  L  Q  K  R  L  D  E  V  S  R  E  L  C  H  460

           1390       1400       1410       1420       1430       1440
             .          .          .          .          .          .
1381 ACGCAGAGCAGCCACGCCAGCCTCCGGGCGGATGCCGAGAAGGCCCAGGAGCAACAGCAG 1440
461  T  Q  S  S  H  A  S  L  R  A  D  A  E  K  A  Q  E  Q  Q  Q  480

           1450       1460       1470       1480       1490       1500
             .          .          .          .          .          .
1441 CAGATGGCCGAGCTGCACAGCAAGTTACAGTCCTCCGAGGCGGAGGTGCGCAGCAAATGC 1500
481  Q  M  A  E  L  H  S  K  L  Q  S  S  E  A  E  V  R  S  K  C  500
```

FIG. 9F

```
          1510       1520       1530       1540       1550       1560
           .          .          .          .          .          .
1501 GAGGAGCTGAGTGGCCTCCACGGGCAGCTCCAGGAGGCCAGGGCAGAGAACTCCCAGCTC 1560
 501  E  E  L  S  G  L  H  G  Q  L  Q  E  A  R  A  E  N  S  Q  L  520

          1570       1580       1590       1600       1610       1620
           .          .          .          .          .          .
1561 ACAGAGAGAATCCGTTCCATTGAGGCCCTGCTGGAGGCGGGCCAGGCGCGGGATGCCCAG 1620
 521  T  E  R  I  R  S  I  E  A  L  L  E  A  G  Q  A  R  D  A  Q  540

          1630       1640       1650       1660       1670       1680
           .          .          .          .          .          .
1621 GACGTCCAGGCCAGCCAGGCGGAGGCTGACCAGCAGCAGACTCGCCTCAAGGAGCTGGAG 1680
 541  D  V  Q  A  S  Q  A  E  A  D  Q  Q  Q  T  R  L  K  E  L  E  560

1690       1700       1710       1720       1730       1740
           .          .          .          .          .          .
1681 TCCCAGGTGTCGGGTCTGGAGAAGGAGGCCATCGAGCTCAGGGAGGCCGTCGAGCAGCAG 1740
 561  S  Q  V  S  G  L  E  K  E  A  I  E  L  R  E  A  V  E  Q  Q  580

          1750       1760       1770       1780       1790       1800
           .          .          .          .          .          .
1741 AAAGTGAAGAACAATGACCTCCGGGAGAAGAACTGGAAGGCCATGGAGGCACTGGCCACG 1800
 581  K  V  K  N  N  D  L  R  E  K  N  W  K  A  M  E  A  L  A  T  600
```

FIG.9G


```
          1810       1820       1830       1840       1850       1860
           .          .          .          .          .          .
1801 GCCGAGCAGGCCTGCAAGGAGAAGCTGCACTCCCTGACCCAGGCCAAGGAGGAATCGGAG 1860
 601  A  E  Q  A  C  K  E  K  L  H  S  L  T  Q  A  K  E  E  S  E  620

          1870       1880       1890       1900       1910       1920
           .          .          .          .          .          .
1861 AAGCAGCTCTGTCTGATTGAGGCGCAGACCATGGAGGCCCTGCTGGCTCTGCTCCCAGAA 1920
 621  K  Q  L  C  L  I  E  A  Q  T  M  E  A  L  L  A  L  L  P  E  640

          1930       1940       1950       1960       1970       1980
           .          .          .          .          .          .
1921 CTCTCTGTCTTGGCACAACAGAATTACACCGAGTGGCTGCAGGATCTCAAAGAGAAAGGC 1980
 641  L  S  V  L  A  Q  Q  N  Y  T  E  W  L  Q  D  L  K  E  K  G  660

          1990       2000       2010       2020       2030       2040
           .          .          .          .          .
1981 CCCACGCTGCTGAAGCACCCGCCAGCTCCCGCGGAGCCCTCCTCGGACCTGGCCTCCAAG 2040
 661  P  T  L  L  K  H  P  P  A  P  A  E  P  S  S  D  L  A  S  K  680

          2050       2060       2070       2080       2090       2100
           .          .          .          .          .          .
2041 TTGAGGGAGGCCGAGGAGACGCAGAGCACACTGCAGGCCGAGTGTGACCAGTACCGCAGC 2100
 681  L  R  E  A  E  E  T  Q  S  T  L  Q  A  E  C  D  Q  Y  R  S  700
```

FIG.9H

```
            2110       2120       2130       2140       2150       2160
              .          .          .          .          .          .
2101 ATCCTGGCGGAGACGGAGGGCATGCTCAGAGACCTGCAGAAGAGCGTGGAGGAGGAGGAG 2160
 701  I  L  A  E  T  E  G  M  L  R  D  L  Q  K  S  V  E  E  E  E  720

            2170       2180       2190       2200       2210       2220
              .          .          .          .          .          .
2161 CAGGTGTGGAGGGCCAAGGTGGGCGCCGCAGAGGAGGAGCTCCAGAAGTCCCGGGTCACA 2220
 721  Q  V  W  R  A  K  V  G  A  A  E  E  E  L  Q  K  S  R  V  T  740

            2230       2240       2250       2260       2270       2280
              .          .          .          .          .          .
2221 GTGAAGCATCTCGAAGAGATTGTAGAGAAGCTAAAAGGAGAACTTGAAAGTTCGGACCAG 2280
 741  V  K  H  L  E  E  I  V  E  K  L  K  G  E  L  E  S  S  D  Q  760

            2290       2300       2310       2320       2330       2340
              .          .          .          .          .          .
2281 GTGAGGGAGCACACGTCGCATTTGGAGGCAGAGCTGGAAAAGCACATGGCGGCCGCCAGC 2340
 761  V  R  E  H  T  S  H  L  E  A  E  L  E  K  H  M  A  A  A  S  780

            2350       2360       2370       2380       2390       2400
              .          .          .          .          .          .
2341 GCCGAGTGCCAGAACTACGCCAAGGAGGTGGCAGGGCTGAGGCAACTTCTCCTAGAATCT 2400
 781  A  E  C  Q  N  Y  A  K  E  V  A  G  L  R  Q  L  L  L  E  S  800
```

FIG.9I


```
            2410       2420       2430       2440       2450       2460
              .          .          .          .          .          .
2401 CAATCTCAGCTCGATGCCGCCAAGAGCGAAGCCCAGAAACAGAGCGATGAGCTTGCCCTG 2460
 801  Q  S  Q  L  D  A  A  K  S  E  A  Q  K  Q  S  D  E  L  A  L  820

         2470       2480       2490       2500       2510       2520
           .          .          .          .          .          .
2461 GTCAGGCAGCAGTTGAGTGAAATGAAGAGCCACGTAGAGGATGGTGACATAGCTGGGGCC 2520
 821  V  R  Q  Q  L  S  E  M  K  S  H  V  E  D  G  D  I  A  G  A  840

2530       2540       2550       2560       2570       2580
  .          .          .          .          .          .
2521 CCAGCTTCCTCCCCAGAGGCGCCCCCAGCCGAGCAGGACCCCGTTCAGCTGAAGACGCAG 2580
 841  P  A  S  S  P  E  A  P  P  A  E  Q  D  P  V  Q  L  K  T  Q  860

            2590       2600       2610       2620       2630       2640
              .          .          .          .          .          .
2581 CTGGAGTGGACAGAAGCCATCCTGGAGGATGAGCAGACACAGCGGCAGAAGCTCATGGCC 2640
 861  L  E  W  T  E  A  I  L  E  D  E  Q  T  Q  R  Q  K  L  M  A  880

            2650       2660       2670       2680       2690       2700
              .          .          .          .          .          .
2641 GAGTTTGAGGAGGCTCAGACCTCGGCATGTCGGTTACAAGAAGAATTGGAGAAGCTCCGC 2700
 881  E  F  E  E  A  Q  T  S  A  C  R  L  Q  E  E  L  E  K  L  R  900
```

FIG.9J

```
          2710       2720       2730       2740       2750       2760
            .          .          .          .          .          .
2701 ACAGCCGGCCCCCTAGAGTCTTCAGAAACAGAGGAGGCCTCACAGCTGAAGGAGAGACTA 2760
 901  T  A  G  P  L  E  S  S  E  T  E  E  A  S  Q  L  K  E  R  L  920

          2770       2780       2790       2800       2810       2820
            .          .          .          .          .          .
2761 GAAAAAGAGAAGAAGTTAACAAGTGACCTGGGGCGCGCCGCCACGAGACTGCAGGAGCTT 2820
 921  E  K  E  K  K  L  T  S  D  L  G  R  A  A  T  R  L  Q  E  L  940

          2830       2840       2850       2860       2870       2880
            .          .          .          .          .          .
2821 CTGAAGACGACCCAGGAGCAGCTGGCAAGGGAGAAGGACACGGTGAAGAAGCTGCAGGAA 2880
 941  L  K  T  T  Q  E  Q  L  A  R  E  K  D  T  V  K  K  L  Q  E  960

          2890       2900       2910       2920       2930
            .          .          .          .          .
2881 CAGCTGGAAAAGGCAGAGGACGGCAGCAGCTCAAAGGAGGGCACCTCTGTCTGA 2934
 961  Q  L  E  K  A  E  D  G  S  S  S  K  E  G  T  S  V  *  977
```

FIG. 9K

HSXCK41 (SEQ ID NOS:19 and 20)

```
            10         20         30         40         50         60
             .          .          .          .          .          .
   1 ATGGCCCAGCTGTTCCTGCCCCTGCTGGCAGCCCTGGTCCTGGCCCAGGCTCCTGCAGCT 60
   1  M  A  Q  L  F  L  P  L  L  A  A  L  V  L  A  Q  A  P  A  A  20

            70         80         90        100        110        120
             .          .          .          .          .          .
  61 TTAGCAGATGTTCTGGAAGGAGACAGCTCAGAGGACCGCGCTTTTCGCGTGCGCATCGCG 120
  21  L  A  D  V  L  E  G  D  S  S  E  D  R  A  F  R  V  R  I  A  40
           130        140        150        160        170        180
             .          .          .          .          .          .
 121 GGCGACGCGCCACTGCAGGGCGTGCTCGGCGGCGCCCTCACCATCCCTTGCCACGTCCAC 180
  41  G  D  A  P  L  Q  G  V  L  G  G  A  L  T  I  P  C  H  V  H  60

           190        200        210        220        230        240
             .          .          .          .          .          .
 181 TACCTGCGGCCACCGCCGAGCCGCCGGGCTGTGCTGGGCTCTCCGCGGGTCAAGTGGACT 240
  61  Y  L  R  P  P  P  S  R  R  A  V  L  G  S  P  R  V  K  W  T  80
```

FIG. 10A

```
          250        260        270        280        290        300
           .          .          .          .          .          .
241 TTCCTGTCCCGGGGCCGGGAGGCAGAGGTGCTGGTGGCGCGGGGAGTGCGCGTCAAGGTG 300
 81  F  L  S  R  G  R  E  A  E  V  L  V  A  R  G  V  R  V  K  V  100

          310        320        330        340        350        360
           .          .          .          .          .          .
301 AACGAGGCCTACCGGTTCCGCGTGGCACTGCCTGCGTACCCAGCGTCGCTCACCGACGTC 360
101  N  E  A  Y  R  F  R  V  A  L  P  A  Y  P  A  S  L  T  D  V  120

          370        380        390        400        410        420
           .          .          .          .          .          .
361 TCCCTGGCGCTGAGCGAGCTGCGCCCCAACGACTCAGGTATCTATCGCTGTGAGGTCCAG 420
121  S  L  A  L  S  E  L  R  P  N  D  S  G  I  Y  R  C  E  V  Q  140

          430        440        450        460        470        480
           .          .          .          .          .          .
421 CACGGCATCGATGACAGCAGCGACGCTGTGGAGTCAAGTCAAAGGTATCCCATCCAGACC 480
141  H  G  I  D  D  S  S  D  A  V  E  S  S  Q  R  Y  P  I  Q  T  160

          490        500        510        520        530        540
           .          .          .          .          .          .
481 CCACGAGAGGCCTGTTACGGAGACATGGATGGCTTCCCCGGGGTCCGGAACTATGGTGTG 540
161  P  R  E  A  C  Y  G  D  M  D  G  F  P  G  V  R  N  Y  G  V  180
```

FIG.10B


```
          550        560        570        580        590        600
           .          .          .          .          .          .
541 GTGGACCCGGATGACCTCTATGATGTGTACTGTTATGCTGAAGACCTAAATGGAGAACTG 600
181  V  D  P  D  D  L  Y  D  V  Y  C  Y  A  E  D  L  N  G  E  L  200

          610        620        630        640        650        660
           .          .          .          .          .          .
601 TTCCTGGGTGACCCTCCAGAGAAGCTGACATTGGAGGAAGCACGGGCGTACTGCCAGGAG 660
201  F  L  G  D  P  P  E  K  L  T  L  E  E  A  R  A  Y  C  Q  E  220

          670        680        690        700        710        720
           .          .          .          .          .          .
661 CGGGGTGCAGAGATTGCCACCACGGGCCAACTGTATGCAGCCTGGGATGGTGGCCTGGAC 720
221  R  G  A  E  I  A  T  T  G  Q  L  Y  A  A  W  D  G  G  L  D  240

          730        740        750        760        770        780
           .          .          .          .          .          .
721 CACTGCAGCCCAGGGTGGCTAGCTGATGGCAGTGTGCGCTACCCCATCGTCACACCCAGC 780
241  H  C  S  P  G  W  L  A  D  G  S  V  R  Y  P  I  V  T  P  S  260
```

FIG.10C

```
            790        800        810        820        830        840
             .          .          .          .          .          .
781 CAGCGCTGTGGTGGGGGCTTGCCTGGTGTCAAGACTCTCTTCCTCTTCCCCAACCAGACT 840
261  Q  R  C  G  G  G  L  P  G  V  K  T  L  F  L  F  P  N  Q  T  280

            850        860        870        880        890        900
             .          .          .          .          .          .
841 GGCTTCCCCAATAAGCACAGCCGCTTCAACGTCTACTGCTTCCGAGACTCGGCCCAGCTT 900
281  G  F  P  N  K  H  S  R  F  N  V  Y  C  F  R  D  S  A  Q  L  300

            910        920        930        940        950        960
             .          .          .          .          .          .
901 CTGCCATCCCTGAGGCCTCCAACCCAGCCTCCAACCCAGCTTGATGGACTAGAGGCTATC 960
301  L  P  S  L  R  P  P  T  Q  P  P  T  Q  L  D  G  L  E  A  I  320
```

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```
            970        980        990       1000       1010       1020
             .          .          .          .          .          .
961 GTCACAGTGACAGAGACCCTGGAGGAACTGCAGCTGCCTCAGGAAGCCACAGAGAGTGAA 1020
321  V  T  V  T  E  T  L  E  E  L  Q  L  P  Q  E  A  T  E  S  E  340

           1030       1040       1050       1060       1070       1080
             .          .          .          .          .          .
1021 TCCCGTGGGGCCATCTACTCCATCCCCATCATGGAGGACGGAGGAGGTGGAAGCTCCACT 1080
341  S  R  G  A  I  Y  S  I  P  I  M  E  D  G  G  G  G  S  S  T  360
```

FIG.10D


```
           1090       1100       1110       1120       1130       1140
             .          .          .          .          .          .
1081 CCAGAAGACCCAGCAGAGGCCCCTAGGACGCTCCTAGAATTTGAAACACAATCCATGGTA 1140
361  P  E  D  P  A  E  A  P  R  T  L  L  E  F  E  T  Q  S  M  V  380

           1150       1160       1170       1180       1190       1200
             .          .          .          .          .          .
1141 CCGCCCACGGGGTTTTCAGAAGAGGAAGGTAAGGCATTGGAGGAAGAAGAGAAATATGAA 1200
381  P  P  T  G  F  S  E  E  E  G  K  A  L  E  E  E  E  K  Y  E  400

           1210       1220       1230       1240       1250       1260
             .          .          .          .          .          .
1201 GATGAAGAAGAGAAAGAGGAGGAAGAAGAAGAGGAGGAGGTGGAGGATGAGGCTCTGTGG 1260
401  D  E  E  E  K  E  E  E  E  E  E  E  V  E  D  E  A  L  W  420
```

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```
           1270       1280       1290       1300       1310       1320
             .          .          .          .          .          .
1261 GCATGGCCCAGCGAGCTCAGCAGCCCGGGCCCTGAGGCCTCTCTCCCCACTGAGCCAGCA 1320
421  A  W  P  S  E  L  S  S  P  G  P  E  A  S  L  P  T  E  P  A  440

           1330       1340       1350       1360       1370       1380
             .          .          .          .          .          .
1321 GCCCAGGAGGAGTCACTCTCCCAGGCGCCAGCAAGGGCAGTCCTGCAGCCTGGTGCATCA 1380
441  A  Q  E  E  S  L  S  Q  A  P  A  R  A  V  L  Q  P  G  A  S  460
```

FIG.10E

```
          1390       1400       1410       1420       1430       1440
            .          .          .          .          .          .
1381 CCACTTCCTGATGGAGAGTCAGAAGCTTCCAGGCCTCCAAGGGTCCATGGACCACCTACT 1440
 461  P  L  P  D  G  E  S  E  A  S  R  P  P  R  V  H  G  P  P  T  480

          1450       1460       1470       1480       1490       1500
            .          .          .          .          .          .
1441 GAGACTCTGCCCACTCCCAGGGAGAGGAACCTAGCATCCCCATCACCTTCCACTCTGGTT 1500
 481  E  T  L  P  T  P  R  E  R  N  L  A  S  P  S  P  S  T  L  V  500

          1510       1520       1530       1540       1550       1560
            .          .          .          .          .          .
1501 GAGGCAAGAGAGGTGGGGGAGGCAACTGGTGGTCCTGAGCTATCTGGGGTCCCTCGAGGG 1560
 501  E  A  R  E  V  G  E  A  T  G  G  P  E  L  S  G  V  P  R  G  520

          1570       1580
            .          .
1561 GGGGCCCGTACCCAATTCGCCCTATAG 1587
 521  G  A  R  T  Q  F  A  L  *  528
```

FIG.10F

HFKFY79 (SEQ ID NOS:21 and 22)

```
            10         20         30         40         50         60
             .          .          .          .          .          .
  1 ATGTCTGCCGACGGGGCAGAGGCTGATGGCAGCACCCAGGTGACAGTGGAAGAACCGGTA 60
  1  M  S  A  D  G  A  E  A  D  G  S  T  Q  V  T  V  E  E  P  V  20

            70         80         90        100        110        120
             .          .          .          .          .          .
 61 CAGCAGCCCAGTGTGGTGGACCGTGTGGCCAGCATGCCTCTGATCAGCTCCACCTGCGAC 120
 21  Q  Q  P  S  V  V  D  R  V  A  S  M  P  L  I  S  S  T  C  D  40

            130        140        150        160        170        180
             .          .          .          .          .          .
121 ATGGTGTCCGCAGCCTATGCCTCCACCAAGGAGAGCTACCCGCACGTCAAGACTGTCTGC 180
 41  M  V  S  A  A  Y  A  S  T  K  E  S  Y  P  H  V  K  T  V  C  60

            190        200        210        220        230        240
             .          .          .          .          .          .
181 GACGCAGCAGAGAAGGGAGTGAGGACCCTCACGGCGGCTGCTGTCAGCGGGGCTCAGCCG 240
 61  D  A  A  E  K  G  V  R  T  L  T  A  A  A  V  S  G  A  Q  P  80
```

FIG.11A

```
          250        260        270        280        290        300
           .          .          .          .          .          .
241 ATCCTCTCCAAGCTGGAGCCCCAGATTGCATCAGCCAGCGAATACGCCCACAGGGGGCTG 300
 81  I  L  S  K  L  E  P  Q  I  A  S  A  S  E  Y  A  H  R  G  L  100

          310        320        330        340        350        360
           .          .          .          .          .          .
301 GACAAGTTGGAGGAGAACCTCCCCATCCTGCAGCAGCCCACGGAGAAGTCCTGGCGGACA 360
101  D  K  L  E  E  N  L  P  I  L  Q  Q  P  T  E  K  S  W  R  T  120

          370        380        390        400        410        420
           .          .          .          .          .          .
361 CAACGACTTGTGTCGTCTAAAGTGTCGGGGCCCAAGAAATGGTGTCTAGCGCCAACGACA 420
121  Q  R  L  V  S  S  K  V  S  G  P  K  K  W  C  L  A  P  T  T  140

          430        440        450        460        470        480
           .          .          .          .          .          .
421 CGGTGGCCACCAATTGTCGGAGCGGTGGACGCGACCCGCGGTGCTGTGCAGAGCGGCGTG 480
141  R  W  P  P  I  V  G  A  V  D  A  T  R  G  A  V  Q  S  G  V  160

          490        500        510        520        530        540
           .          .          .          .          .          .
481 GACAAGACAAAGTCCGTAGTGACCGGCGGCGTCCAATCGGTCATGGGCTCCCGCTTGGGC 540
161  D  K  T  K  S  V  V  T  G  G  V  Q  S  V  M  G  S  R  L  G  180
```

FIG.11B


```
          550        560        570        580        590        600
           .          .          .          .          .          .
541 GGCACGAGGCTGAGTGGGGTCGACACGGTGCTGGGGAAGTCGGAGGAGTGGGCGGACAAC 600
181  G  T  R  L  S  G  V  D  T  V  L  G  K  S  E  E  W  A  D  N  200

          610        620        630        640        650        660
           .          .          .          .          .          .
601 CACCTGCCCCTTACGGATGCCGAACTGGCCCGCATCGCCACATCCCTGGATGGCTTCGAC 660
201  H  L  P  L  T  D  A  E  L  A  R  I  A  T  S  L  D  G  F  D  220

          670        680        690        700        710        720
           .          .          .          .          .          .
661 GTCGCGTCCGTGCAGCAGCAGCGGCAGGAACAGAGCTACTTCGTACGTCTGGGCTCCCTG 720
221  V  A  S  V  Q  Q  Q  R  Q  E  Q  S  Y  F  V  R  L  G  S  L  240

          730        740        750        760        770        780
           .          .          .          .          .          .
721 TCGGAGAGGCTGCGGCAGCACGCCTATGAGCACTCGCTGGGCAAGCTTCGAGCCACCAAG 780
241  S  E  R  L  R  Q  H  A  Y  E  H  S  L  G  K  L  R  A  T  K  260
```

FIG.11C

```
          790        800        810        820        830        840
           .          .          .          .          .          .
781 CAGAGGGCACAGGAGGCTCTGCTGCAGCTGTCGCAGGCCCTAAGCCTGATGGAAACTGTC 840
261  Q  R  A  Q  E  A  L  L  Q  L  S  Q  A  L  S  L  M  E  T  V  280

          850        860        870        880        890        900
           .          .          .          .          .          .
841 AAGCAAGGCGTTGATCAGAAGCTGGTGGAAGGCCAGGAGAAGCTGCACCAGATGTGGCTC 900
281  K  Q  G  V  D  Q  K  L  V  E  G  Q  E  K  L  H  Q  M  W  L  300

          910        920        930        940        950        960
           .          .          .          .          .          .
901 AGCTGGAACCAGAAGCAACTCCAGGGCCCCGAGAAGGAGCCGCCCAAGCCAGAGCAGGTC 960
301  S  W  N  Q  K  Q  L  Q  G  P  E  K  E  P  P  K  P  E  Q  V  320

          970        980        990       1000       1010       1020
           .          .          .          .          .          .
961 GAGTCCCGGGCGCTCACCATGTTCCGGGACATTGCCCAGCAACTGCAGGCCACCTGTACC 1020
321  E  S  R  A  L  T  M  F  R  D  I  A  Q  Q  L  Q  A  T  C  T  340

         1030       1040       1050       1060       1070       1080
           .          .          .          .          .          .
1021 TCCCTGGGGTCCAGCATTCAGGGCCTCCCCACCAATGTGAAGGACCAGGTGCAGCAGGCC 1080
341  S  L  G  S  S  I  Q  G  L  P  T  N  V  K  D  Q  V  Q  Q  A  360
```

FIG. 11D


```
         1090       1100       1110       1120       1130       1140
           .          .          .          .          .          .
1081 CGCCGCCAGGTGGATGACCTCCATGCCACGTTTTCCAACATCCACTCCTTCCAGGACCTG 1140
361  R  R  Q  V  D  D  L  H  A  T  F  S  N  I  H  S  F  Q  D  L  380

         1150       1160       1170       1180       1190       1200
           .          .          .          .          .          .
1141 TCCAGCAACAATTCTGGCCCAGAGCCGTTAGTGTTCGCCAGCGCCCGCGAGGCCCTGGAC 1200
381  S  S  N  N  S  G  P  E  P  L  V  F  A  S  A  R  E  A  L  D  400

         1210       1220       1230       1240       1250       1260
           .          .          .          .          .          .
1201 CACATGGTGGGAATGATGTGGCCCACAACTCCCCTGTTTCCATGGTCTCTGTTGGGGACC 1260
401  H  M  V  G  M  M  W  P  T  T  P  L  F  P  W  S  L  L  G  T  420

         1270       1280       1290       1300       1310       1320
           .          .          .          .          .          .
1261 CTTTTGCCCCTTGTGATTCACTCGAGAAAGCCCCCAGAGGCAAAACAATTTTGGGGACAG 1320
421  L  L  P  L  V  I  H  S  R  K  P  P  E  A  K  Q  F  W  G  Q  440

         1330       1340       1350
           .          .          .
1321 GAGAGGACTCAGCGGGCTCCCGTCTCTATAATGCAGTGA 1359
441  E  R  T  Q  R  A  P  V  S  I  M  Q  *  452
```

FIG. 11E

HAICH28 (SEQ ID NOS:23 and 24)

```
                10        20        30        40        50        60
                .         .         .         .         .         .
  1 ATGGCGACCCCAGCCTCGGCCCCAGACACACGGGCTCTGGTGGCAGACTTTGTAGGTTAT 60
  1 M  A  T  P  A  S  A  P  D  T  R  A  L  V  A  D  F  V  G  Y  20

                70        80        90       100       110       120
                .         .         .         .         .         .
 61 AAGCTGAGGCAGAAGGGTTATGTCTGTGGAGCTGGCCCCGGGGAGGGCCCAGCAGCTGAC 120
 21 K  L  R  Q  K  G  Y  V  C  G  A  G  P  G  E  G  P  A  A  D  40

               130       140       150       160       170       180
                .         .         .         .         .         .
121 CCGCTGCACCAAGCCATGCGGGCAGCTGGAGATGAGTTCGAGACCCGCTTCCGGCGCACC 180
 41 P  L  H  Q  A  M  R  A  A  G  D  E  F  E  T  R  F  R  R  T  60

               190       200       210       220       230       240
                .         .         .         .         .         .
181 TTCTCTGATCTGGCGGCTCAGCTGCATGTGACCCCAGGCTCAGCCCAACAACGCTTCACC 240
 61 F  S  D  L  A  A  Q  L  H  V  T  P  G  S  A  Q  Q  R  F  T  80
```

FIG.12A


```
               250       260       270       280       290       300
                .         .         .         .         .         .
241 CAGGTCTCCGATGAACTTTTTCAAGGGGGCCCCAACTGGGGCCGCCTTGTAGCCTTCTTT 300
 81 Q  V  S  D  E  L  F  Q  G  G  P  N  W  G  R  L  V  A  F  F  100

               310       320       330       340       350       360
                .         .         .         .         .         .
301 GTCTTTGGGGCTGCACTGTGTGCTGAGAGTGTCAACAAGGAGATGGAACCACTGGTGGGA 360
101 V  F  G  A  A  L  C  A  E  S  V  N  K  E  M  E  P  L  V  G  120

               370       380       390       400       410       420
                .         .         .         .         .         .
361 CAAGTGCAGGAGTGGATGGTGGCCTACCTGGAGACGCGGCTGGCTGACTGGATCCACAGC 420
121 Q  V  Q  E  W  M  V  A  Y  L  E  T  R  L  A  D  W  I  H  S  140

               430       440       450       460       470       480
                .         .         .         .         .         .
421 AGTGGGGGCTGGTTATCCCAGATCACTGAAGCTGAGATGGCTGATGAAGTAATTTGCAGT 480
141 S  G  G  W  L  S  Q  I  T  E  A  E  M  A  D  E  V  I  C  S  160

               490       500       510       520       530       540
                .         .         .         .         .         .
481 GAAATTTTAAGCGACTGTGACTCTGCTGCAAGTTCCCCAGATCTTGAGGAGCTGGAAGCT 540
161 E  I  L  S  D  C  D  S  A  A  S  S  P  D  L  E  E  L  E  A  180
```

FIG.12B

```
          550        560        570        580        590        600
           .          .          .          .          .          .
541 ATCAAAGCTCGAGTCAGGGAGATGGAGGAAGAAGCTGAGAAGCTAAAGGAGCTACAGAAC 600
181  I  K  A  R  V  R  E  M  E  E  E  A  E  K  L  K  E  L  Q  N  200

          610        620        630        640        650        660
           .          .          .          .          .          .
601 GAGGTAGAGAAGCAGATGAATATGAGTCCACCTCCAGGCAATGCTGGCCCGGTGATCATG 660
201  E  V  E  K  Q  M  N  M  S  P  P  P  G  N  A  G  P  V  I  M  220

       670        680        690        700        710        720
        .          .          .          .          .          .
661 TCCATTGAGGAGAAGATGGAGGCTGATGCCCGTTCCATCTATGTTGGCAATGTGGACTAT 720
221  S  I  E  E  K  M  E  A  D  A  R  S  I  Y  V  G  N  V  D  Y  240

          730        740        750        760        770        780
           .          .          .          .          .          .
721 GGTGCAACAGCAGAAGAGCTGGAAGCTCACTTTCATGGCTGTGGTTCAGTCAACCGTGTT 780
241  G  A  T  A  E  E  L  E  A  H  F  H  G  C  G  S  V  N  R  V  260

          790        800        810        820        830        840
           .          .          .          .          .          .
781 ACCATACTGTGTGACAAATTTAGTGGCCATCCCAAAGGGTTTGCGTATATAGAGTTCTCA 840
261  T  I  L  C  D  K  F  S  G  H  P  K  G  F  A  Y  I  E  F  S  280
```

FIG.12C


```
          850        860        870        880        890        900
           .          .          .          .          .          .
841 GACAAAGAGTCAGTGAGGACTTCCTTGGCCTTAGATGAGTCCCTATTTAGAGGAAGGCAA 900
281  D  K  E  S  V  R  T  S  L  A  L  D  E  S  L  F  R  G  R  Q  300

          910        920        930        940        950        960
           .          .          .          .          .          .
901 ATCAAGGTGATCCCAAAACGAACCAACAGACCAGGCATCAGCACAACAGACCGGGGTTTT 960
301  I  K  V  I  P  K  R  T  N  R  P  G  I  S  T  T  D  R  G  F  320

          970        980        990       1000       1010       1020
           .          .          .          .          .          .
961 CCACGAGCCCCGTACCGCGCCCGGACCACCAACTACAACAGCTCCCGCTCTCGATTCTAC 1020
321  P  R  A  P  Y  R  A  R  T  T  N  Y  N  S  S  R  S  R  F  Y  340

         1030       1040       1050       1060       1070       1080
           .          .          .          .          .          .
1021 AGTGGTTTTAACAGCAGGCCCCGGGGTCGCGTCTACAGGGGCCGGGCTAGAGCGACATCA 1080
341  S  G  F  N  S  R  P  R  G  R  V  Y  R  G  R  A  R  A  T  S  360

         1090
           .
1081 TGGTATTCCCCTTACTAA 1098
361  W  Y  S  P  Y  *  365
```

FIG.12D

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(57) Abstract

The present invention relates to novel human proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells and recombinant methods for producing the proteins of the invention. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human secreted proteins.

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BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

RNSDOCID: <WO 9831800A1 >

INTERNATIONAL SEARCH REPORT

Interna[illegible]al Application No
PCT/US 98/00960

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N5/10 C07K14/47 C12Q1/68 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	T. FUJIWARA: "Otsuka cDNA project" EMBL SEQUENCE DATABASE, 4 October 1996, HEIDELBERG, FRG, XP002070248 Human placenta cDNA 5'-end GEN-548B01; Accession no. C17482; ---	1,2,9-14
X	H. FRIESS ET AL.: "A pancreatic cancer-specific expression profile" EMBL SEQUENCE DATABASE, 20 May 1996, HEIDELBERG, FRG, XP002070249 Human clone xs255 mRNA sequence; Acession no. U46281; ---	1,2,9-14
A	WO 92 04376 A (HEART RES INST) 19 March 1992 see the whole document --- -/--	1-15

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 July 1998	09. 10. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	HORNIG H.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna[illegible]al Application No
PCT/US 98/00960

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 263 072 A (CIBA GEIGY AG) 6 April 1988 see the whole document ---	1-15
A	WO 90 03394 A (ROUSSEL UCLAF) 5 April 1990 see the whole document ---	1-15
A	WO 95 17506 A (HARVARD COLLEGE) 29 June 1995 see the whole document ---	1-15
A	WO 95 03328 A (BOEHRINGER MANNHEIM GMBH ;BOGDAHN ULRICH (DE); BUETTNER REINHARD () 2 February 1995 cited in the application see the whole document ---	1-15
A	WO 90 14432 A (GENETICS INST) 29 November 1990 see the whole document ---	1-15
A	WO 96 17925 A (IMMUNEX CORP) 13 June 1996 see the whole document ---	1-15
A	US 5 536 637 A (JACOBS KENNETH) 16 July 1996 cited in the application see the whole document -----	1-15

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In' itional application No.
PCT/US 98/00960

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see further information sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see extra sheet, subject 1.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: (1-15) partially

An isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide selected from SEQ ID no.2; said nucleotide sequence which is at least 95% identical to a nucleotide sequence selectde from SEQ ID no.1; an isolated nucleic acid molecule which hybridizes under stringent conditions to said nucleic acid molecule; a method of making a recombinant vector comprising said nucleic acid molecule; a recombinant host cell comprising said vector; a method of making an isolated polypeptide od SEQ ID no. 2; an isolated polypeptide of SEQ ID no.2; an isolated antibody capable of specifically binding to said polypeptide;

2. Claims: (1-15) partially

Idem as subject 1 but limited to SEQ ID nos. 3 and 4;

3. Claims: (1-15) partially

Idem as subject 1 but limited to SEQ ID nos. 5 and 6;

4. Claims: (1-15) partially

Idem as subject 1 but limited to SEQ ID nos. 7 and 8;

5. Claims: (1-15) partially

Idem as subject 1 but limited to SEQ ID nos. 9 and 10;

6. Claims: (1-15) partially

Idem as subject 1 but limited to SEQ ID nos. 11 and 12;

7. Claims: (1-15) partially

Idem as subject 1 but limited to SEQ ID nos. 13 and 14;

8. Claims: (1-15) partially

Idem as subject 1 but limited to SEQ ID nos. 15 and 16;

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

9. Claims: (1-15) partially

Idem as subject 1 but limited to SEQ ID nos. 17 and 18;

10. Claims: (1-15) partially

Idem as subject 1 but limited to SEQ ID nos. 19 and 20;

11. Claims: (1-15) partially

Idem as subject 1 but limited to SEQ ID nos. 21 and 22;

12. Claims: (1-15) partially

Idem as subject 1 but limited to SEQ ID nos. 23 and 24;

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna al Application No
PCT/US 98/00960

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 8504091 A	30-03-92
		EP 0550506 A	14-07-93
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna al Application No
PCT/US 98/00960

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